

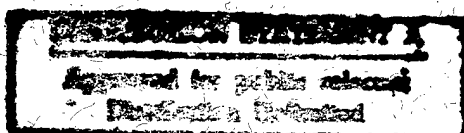
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REPLY TO  
ATTENTION OF

DEPARTMENT OF THE ARMY  
WATERWAYS EXPERIMENT STATION, CORPS OF ENGINEERS  
3909 HALLS FERRY ROAD  
VICKSBURG, MISSISSIPPI 39180-6199

August 21, 1998

Environmental Laboratory

The enclosed Proceedings of the U.S. Army Corps of Engineers Aquatic Plant Control Research Program (APCRP) was published as a special section in the Journal of Aquatic Plant Management (Volume 36: 23-87, 1998), and is being provided in this format as a technology transfer service of the APCRP. The research reported in these proceedings was presented at the 37<sup>th</sup> Annual Meeting of the Aquatic Plant Management Society, Inc. (APMS) held in Ft. Myers, Florida, July 13-16, 1997.

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The publication of this special section was made possible through the volunteer efforts of Dr. John D. Madsen, Editor, and the APMS Board of Directors.

Sincerely,

JOHN W. BARKO

John W. Barko, PhD  
Director  
Center for Aquatic Plant Research and Technology

Enclosure

# An Overview of the Aquatic Plant Control Research Program

ROBERT C. GUNKEL, JR.<sup>1</sup> AND JOHN W. BARKO<sup>1</sup>

## ABSTRACT

The U.S. Army Corps of Engineers (CE) Aquatic Plant Control Research Program (APCRP) is the Nation's only federally authorized research program directed to develop technology for the management of non-indigenous aquatic plant species. The APCRP is designed to provide effective, economical, and environmentally compatible methods for assessing and managing problem aquatic plants that interfere with the valued uses of the waterways of the United States. Research efforts are currently focused on the development of advanced management strategies and applications for the submersed aquatic plants, hydrilla (*Hydrilla verticillata* (L.f.) Royle) and Eurasian watermilfoil (*Myriophyllum spicatum* L.). The APCRP is committed to the development, transfer, and implementation of aquatic plant management technologies, and will continue to lead the Nation in the future.

**Key words:** aquatic plant management, aquatic plant ecology, biological control, chemical control, hydrilla, Eurasian watermilfoil.

## PROGRAM HISTORY

During the late 1880's and early 1890's the non-indigenous aquatic plant, waterhyacinth (*Eichhornia crassipes* (Mart.) Solms), rapidly infested the waters of Florida and Louisiana. The expanding populations of waterhyacinth obstructed commercial river traffic, leading Congress to approve the River and Harbor Act of 1899. This act authorized the U.S. Army Corps of Engineers (CE) to remove waterhyacinth in the navigable waters of Florida, Louisiana, Texas, Mississippi, and Alabama. Thus, initial responsibilities for aquatic plant management by the CE were established in 1899.

By the mid-1940's, another non-indigenous aquatic plant, alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb.), had infested the waters of the southeastern United States. In view of the magnitude of this new aquatic plant problem, Congress revised the River and Harbor Act in 1958 (Section 104, Public Law 85-500). This newly approved act authorized the CE to proceed with a comprehensive project for the control and progressive eradication of alligatorweed, in addition to waterhyacinth and other noxious aquatic plants, in the waters of Florida, Louisiana, Texas, Mississippi, Alabama, North Carolina, South Carolina, and Georgia. In recognition of the value of scientific research to solving problems, Congress at the same time included provisions for research

directed toward the development of the most effective and economic control methods. This project, known as the Expanded Project for Aquatic Plant Control, was initiated in 1959 for a five-year period.

In 1965, a report on the results of the Expanded Project was submitted to Congress recommending that the "project" approach should be expanded to a nationwide "program." As a result, Section 302 of the River and Harbor Act of 1965 (Public Law 89-298) authorized the CE to provide an expanded program of research directed toward the control and progressive eradication of waterhyacinth, alligatorweed, and other noxious aquatic plants in the waters of the entire United States.

In early 1975, the CE Headquarters designated the U.S. Army Engineer Waterways Experiment Station (WES) in Vicksburg, Mississippi, as the Corps' lead laboratory for aquatic plant research. The Aquatic Plant Control Research Program (APCRP) was established with responsibility for the management of the National research program. Today, activities of the APCRP are conducted under the Center for Aquatic Plant Research and Technology (CAPRT) at the WES. The CAPRT was established in August 1993 to provide administrative leadership, coordination, and facilitation of all aquatic plant research and transfer of technology.

## PROGRAM IMPLEMENTATION

The APCRP is the Nation's only federally authorized research program for aquatic plant management, and is nationally-recognized as the leader in aquatic plant management research and technology development. The research program is designed to provide effective, economical, and environmentally compatible technology for the assessment and management of aquatic plant problems of major economic significance in waters of the United States. The transfer and implementation of technologies developed under the APCRP are provided through a variety of media (e.g., user manuals, instruction reports, journal articles, technical notes, information bulletins, field demonstrations, computer-based information systems, simulation tools, and technical workshops and training courses). The coordination of research and technology development is continuous, and maintained with a national network of cooperators from other Federal agencies, state agencies, local governments, universities, national and regional organizations, and private industry.

For more than 20 years the APCRP has provided research and technology development in the areas of: 1) chemical, biological, mechanical, and integrated control; 2) ecological studies; and 3) simulation and modeling. Presently, the major focus of the program is on the management of the

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non-indigenous submersed aquatic plants, hydrilla and Eurasian watermilfoil. Research efforts are organized into four separate, but technically integrated, technology development areas: Biological Control; Chemical Control; Ecological Assessment; and Management Strategies and Applications.

## **TECHNOLOGY DEVELOPMENT**

### **Biological Control**

In the Biological Control technology development area of the APCRP, techniques are being developed that incorporate biological agents in the management of non-indigenous aquatic plants. Biological agents are acquired through domestic and foreign exploration. A portion of the work for importation and quarantine of "introduced" biological agents is being accomplished through a Cooperative Agreement with the U.S. Department of Agriculture, Agricultural Research Service.

Presently, Biological Control research is focused on the development of techniques that utilize insects, microbial pathogens, or a combination of the two, in the management of hydrilla and Eurasian watermilfoil. Insect biocontrol studies involve the identification of potential insect agents, development of appropriate release and establishment procedures, and evaluations of effectiveness. Plant microbial studies involve the identification, evaluation, and formulation of endemic and exotic pathogens.

### **Chemical Control**

Research in the Chemical Control technology development area of the APCRP is directed toward the development of methods to improve the use of aquatic plant herbicides and plant growth regulators. A primary objective of this research is to evaluate lower herbicide use rates, resulting in application techniques with reduced application costs and improved environmental compatibility. Currently in the Chemical Control area, techniques are being developed to ensure species-selectivity and improved delivery of herbicides to target plants. A cooperative relationship with the chemical industry and the U.S. Environmental Protection Agency has been established to facilitate information transfer.

The species-selective properties of various aquatic herbicides are being evaluated to allow for the removal of non-indigenous aquatic plants, while minimizing the impacts on native aquatic plants. Advanced aquatic herbicide delivery systems are being evaluated for use with existing and new herbicide formulations to improve submersed application delivery and maximize the efficacy on target plants, while providing improved environmental compatibility.

### **Ecological Assessment**

The Ecological Assessment technology development area provides the basic knowledge required to understand the biology of aquatic plants and their role in the aquatic environment. Research in this area is designed to allow for a better understanding of the growth and spread of problem aquatic plants under various environmental conditions. This knowledge is essential for designing aquatic plant manage-

ment plans that are both effective and environmentally compatible. In addition, research on the establishment of native plants is being conducted. Present studies focus on environmental factors influencing plant propagule production and success, establishment of desirable native aquatic plants, environmental factors influencing plant invasions, and effects of aquatic plants on habitat conditions.

Studies on environmental factors influencing propagule production and success focus on the effects of key environmental variables on the number, size, and growth of plant propagules. Ongoing evaluation and development of techniques for establishing native aquatic plants will provide guidance to natural resource managers for successfully establishing diverse communities of desirable native aquatic plants. Investigations of aquatic plant invasions presently focus on environmental factors related to invasion success. In combination with the foregoing efforts, studies directed ultimately toward ecosystem restoration presently focus on the effects of different types of aquatic plants on habitat conditions.

### **Management Strategies and Applications**

Activities in the Management Strategies and Applications technology development area are directed toward integration and adaptation of technologies to the needs of aquatic plant management; for example, integrated use of herbicides and pathogens is currently being evaluated to determine possible additive, synergistic, or antagonistic relationships in aquatic plant management. An aquatic plant management "strategy planner," currently under development will soon provide a CD-based information system that contains all available APCRP simulation models. As part of the "strategy planner" an expert system for aquatic plant identification and various types of control will be provided. In addition, hyperlinked textual information on aquatic plant ecology, sampling, and evaluation will be made available.

## **CONCLUDING REMARKS**

Since 1975, the staff and researchers of the APCRP have developed a research program that is recognized as the National leader in all areas of aquatic plant research and technology development. The continued spread of non-indigenous aquatic plant species to new areas and the introduction of new non-indigenous aquatic plants demands that research continues to provide effective technology for aquatic plant management. For the foreseeable future, the staff and researchers of the APCRP are committed to lead the Nation in the development of technologies needed for the consistent management of non-indigenous aquatic plant species. Technology development and transfer will be continued for chemical, biological, and integrated controls, as well as for ecosystem restoration and the establishment of native aquatic plants.

## **ACKNOWLEDGMENTS**

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# Overview of the Ecological Assessment Technology Area

JOHN D. MADSEN<sup>1</sup>

## ABSTRACT

Research in the Ecological Assessment Technology Area of the Aquatic Plant Control Research Program has contributed extensively to understanding the basic and applied ecology of nonnative and native aquatic plants. Continuing ecological research in this technology area strives to be relevant to aquatic plant management through a focus on the following areas; 1) improving the effectiveness of management techniques, 2) evaluating the effectiveness of management techniques, 3) evaluating the impacts of management techniques on nontarget species and environmental quality, and 4) preventing new infestations of nonnative aquatic plants. While both current and future planned research falls within these areas, specific research needs for the future have been identified in the areas of 1) developing standard aquatic plant quantification methods, 2) developing protocols for identifying the potential of new species to create nuisance problems, and 3) developing spatial databases and spatial models of plant distribution.

**Key words:** aquatic plant ecology, efficacy, management, ecological impacts, aquatic macrophyte ecology.

## INTRODUCTION

Nonnative aquatic plant species have been a multifaceted problem for water resources in the United States throughout this century (Gallagher and Haller 1990). All of the contiguous 48 states have unwelcome nonnative aquatic plant species that, in some measure, create water resource problems. The principal nonnative problem species in the United States are hydrilla (*Hydrilla verticillata* (L.f.) Royle), Eurasian watermilfoil (*Myriophyllum spicatum* L.), and waterhyacinth (*Eichhornia crassipes* (Mart.) Solms, Madsen 1997a). Many other species pose regionally-important problems, or are increasing in their range, including water lettuce (*Pistia stratiotes* L.), waterchestnut (*Trapa natans* L.) and Brazilian elodea (*Egeria densa* Planch.). As with terrestrial nonnative plant species, aquatic species constitute a major threat to the utility and integrity of natural ecosystems.

Water resource problems of most proximal interest for government agencies are the nuisances affecting navigation, flood control, hydroelectric power generation, mosquito-borne diseases and water supply. Other direct uses of water resources impeded by noxious plant growths include recreational use and shoreline property value.

In addition, other effects of these species on natural and manmade aquatic ecosystems have emerged in recent decades. The dense growths created by nonnative aquatic plants can degrade water quality, including decreased oxygen concentrations in water (Frodge et al. 1995), increased nutrient release from sediments (Seki et al. 1979, Frodge et al. 1991), and nutrient release from decomposing plant material (Smith and Adams 1986). Dense monospecific stands of nonnative species suppress the diversity and abundance of native plant species (Madsen et al. 1991), including rare, threatened and endangered plant species. Although aquatic plants are a superior substrate to bare bottom for macroinvertebrates, mixed stands of native plants are typically better as habitat than dense monospecific stands of nonnative species (Keast 1984, Hanson 1990). As the proportion of littoral zone taken up by dense monospecific stands of nonnative species increases, the predator/prey balance of lake ecosystems will shift, which may result in reduced size of predatory fish, including desirable game fish (Wiley et al. 1984, Lillie and Budd 1992). The shift from no plants or from native plants to dense stands of nonnative species will have substantial impacts on human use and ecological balances in aquatic ecosystems.

Recent research indicates that active management of nonnative species not only reduces the nuisance aspect to human activities, but can also restore native plant diversity and abundance, regardless of whether management approaches use herbicides (Getsinger et al. 1997), mechanical control (Eichler et al. 1993), or physical control (Eichler et al. 1995). Selective biocontrol approaches presumably will also preserve or enhance native species diversity and abundance.

## IMPORTANCE OF ECOLOGICAL RESEARCH

Ecological research must be an integral component of ongoing aquatic plant control research in four areas: 1) improving the effectiveness of management techniques, 2) evaluating the effectiveness of management techniques, 3) evaluating the impacts of management techniques, and 4) preventing new infestations.

Ecological research can improve the effectiveness of management techniques through a better knowledge of both the target invasive species and nontarget species. For instance, studies of the seasonal growth cycle of nonnative species might reveal when they are most susceptible to control (Madsen 1997b). Increased knowledge of the nontarget species might improve timing of management techniques to minimize impacts on nontarget plants. A better understanding of the life cycle of target plant species might also explain failures in management programs and suggest system management approaches to reduce the reestablishment of nonnative species.

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Ecological studies are also needed to address aspects of aquatic plant management not traditionally included in cost/benefit analyses. In a time of shrinking economic resources to manage a growing problem, economic cost/benefit analyses have become critical elements for operational aquatic plant management programs. Although the economic cost of treating an acre of aquatic plants can be computed without considering ecological factors, this type of valuation gives no insight into how effective the technique is and how long the effects of the management technique will remain. Evaluations of management effectiveness should be considered as part of a cost/benefit analysis.

A growing concern of natural resource agencies is the impacts of aquatic plant management activities on the aquatic ecosystems they manage and regulate. Ecological studies of aquatic plant management activities will provide an objective and realistic look at the true impacts of management on aquatic resources. All management approaches have some impact on the ecosystem. Many have both positive and negative impacts that must be weighed in choosing an appropriate management technique. The lack of quantitative data on management impacts to aquatic communities hampers an objective evaluation.

Ecological research not only assists in developing plans to prevent the spread of a known nonnative nuisance species to new sites, but may also assist in predicting new species that may become a problem in the future.

## **HISTORY OF ECOLOGICAL ASSESSMENT AREA**

The Ecological Assessment Area of the Aquatic Plant Control Research Program (APCRP) has been a national leader in studies of the basic ecology of aquatic plants. Studies focused on factors limiting aquatic plant distribution and abundance are among the most cited aquatic plant literature, including studies on limitation by sediment nutrients (Barko and Smart 1981b), aqueous nutrients (Barko 1982), salinity (Twilley and Barko 1990), water temperature (Barko et al. 1982) and light availability (Barko and Smart 1981a). In addition, previous research has included studies of water movement (James and Barko 1991), competition between native and nonnative aquatic plants (Smart et al. 1994), and phenological studies of aquatic plants (Luu and Getsinger 1990, Madsen 1997b).

Several simulation models have been developed to assist the aquatic plant manager in planning and implementing management strategies. These include models for stocking grass carp (AMUR STOCK, Stewart and Boyd 1994), herbicide dissipation (HERBICIDE, Stewart 1994), harvester operations (HARVEST, Sabol 1983), and insect biocontrol agents on waterhyacinth (INSECT, Stewart and Boyd 1992). A plant growth model was recently developed for the growth of hydrilla (HYDRIL, Best and Boyd 1996) and one is currently under development for Eurasian watermilfoil (MILFO). Ecological studies have been an important component of model development.

## **CURRENT RESEARCH AREAS**

There are currently four research topics within the Ecological Assessment area:

1) Factors Influencing Propagule Production and Success in Submersed Macrophytes. Studies in this area are designed

to assess the influences of environmental variables on the number, size, and probability of success of plant propagules, and the tuber or seed stage which may be the most vulnerable point in the life cycle of some plants. Research to date has evaluated the effects of temperature and growth stage (McFarland and Barko 1994) and sediment nutrient availability (McFarland and Barko 1996, Rogers et al. 1996) on propagules of selected submersed species. The establishment potential of regenerative fragments relative to their physiological status has also been investigated (McFarland and Barko 1996). Studies are currently being conducted to examine propagule emergence and growth relative to depth of burial in different sediments.

2) Factors Influencing Submersed Plants Invasions and Declines. This research area has investigated causes of nuisance plant population declines, predominantly Eurasian watermilfoil (Smith and Barko 1990, 1996). In recent years, it has sought to identify environmental factors related to successful invasions.

3) Coordination of Control Tactics with Phenological Events of Aquatic Plants. Carbohydrate storage patterns of waterhyacinth (Luu and Getsinger 1990), hydrilla and Eurasian watermilfoil (Madsen 1997b) were evaluated over several annual cycles to determine points at which these species might be most susceptible to control. Distinct low points in carbohydrate storage were identified for Eurasian watermilfoil and hydrilla that might be exploited for management.

4) Techniques for Establishing Native Aquatic Plants. In this research area, native plant establishment is studied as a mechanism to mitigate aquatic plant management operations, fill an empty niche in new reservoir systems without plants, and improve fish habitat in surface water resources (Smart et al. 1996).

## **FUTURE RESEARCH DIRECTIONS**

The future research directions stem directly from the importance of ecological studies cited above.

Improving the effectiveness of management techniques will require research on all major aspects of plant allocation and propagation, phenology, seasonal studies, and whole-plant physiology. In addition, it may require whole-plant studies of why management techniques work, such as biocontrol and mechanical management techniques.

Evaluating the effectiveness of management techniques will require monitoring operational implementation of all management techniques, including biological controls, herbicides, mechanical and physical control approaches. To perform these studies, research needs to be done to develop standardized plant community quantification techniques. This will allow us to utilize data collected by cooperators or others across the country by ensuring that the data are directly comparable. As part of field evaluation studies, data may also be collected on desirable native plant communities to evaluate the impacts of management on nontarget species.

Evaluating the ecological effects of management techniques may combine both field studies of operational efforts, as well as controlled pond and mesocosm studies of biological, chemical, mechanical and physical techniques and their impacts on nontarget plants, animals, and water quality.

Efforts to minimize the spread of nonnative species and to mitigate for management operations will require some additional research on the restoration and creation of littoral zone habitats dominated by desirable native species.

Prevention of new introductions and the spread of existing problem species to new areas will require research on evaluation techniques for new potential nonnative invaders. The development of spatial databases and spatial models for aquatic plant distribution and management will further aid the development of management planning, to direct resources to the most potentially vulnerable areas.

Lastly, the needs of technology transfer will require further development of computer-based decision support tools, integrated between technology areas, to assist in the planning and implementation of operational aquatic plant management.

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# Predicting Invasion Success of Eurasian Watermilfoil

JOHN D. MADSEN<sup>1</sup>

## ABSTRACT

A better understanding of factors related to invasion and colonization success of exotic species might improve both the planning and implementation of management for invasions in new areas. Data from lakes containing Eurasian watermilfoil were evaluated to compare the extent of Eurasian watermilfoil dominance to common limnological parameters. The best predictors of Eurasian watermilfoil dominance were water column total phosphorus and Carlson's Trophic State Index. This analysis corroborates observations that Eurasian watermilfoil appears most abundant in mesotrophic lakes and moderately eutrophic lakes.

**Key words:** *Myriophyllum spicatum*, nonindigenous aquatic plant, exotic aquatic macrophyte, colonization.

## INTRODUCTION

Littoral zone plants are an important component of the lake ecosystem (Ozimek et al. 1990), providing food and habitat for macroinvertebrates and fish (Cyr and Downing 1988, Savino and Stein 1989), stabilizing bottom sediments and binding nutrients (Maceina et al. 1992), and reducing turbidity in the water column by increasing sedimentation rates (Petticrew and Kalff 1992). Nevertheless, the introduction of nonindigenous aquatic plants into littoral zone environments may alter the complex web of biotic and abiotic interactions. Dense stands of some mat-forming plant species reduce oxygen exchange, deplete available dissolved oxygen, and increase water temperatures, and increase internal loading rates of nutrients (Frodge et al. 1991, 1995, Seki et al. 1979). Dense canopies formed by some nonindigenous species reduce native plant diversity and abundance (Madsen et al. 1991). The reduction of habitat complexity results in reduced macroinvertebrate diversity and abundance (Krull 1970, Keast 1984), and also reduces growth of fishes (Lillie and Budd 1992). The advent of nonindigenous plant species is not only deleterious to human use of aquatic systems but detrimental to the native ecosystem.

Eurasian watermilfoil (*Myriophyllum spicatum* L.) was first introduced to the United States in the 1940's (Couch and Nelson 1985). Presently, it is found in 44 of the lower 48 states<sup>2</sup> and several Canadian provinces from Quebec to British Columbia (Aiken et al. 1979, Couch and Nelson 1985).

Eurasian watermilfoil is a perennial herbaceous submersed plant which forms a dense canopy of branches at the surface (Aiken et al. 1979, Smith and Barko 1990). Eurasian watermilfoil spreads from one lake to another by mass flow of water and by accidental introduction on boats and boat trailers (Aiken et al. 1979, Newroth 1993). Spread between lakes and within lakes is predominantly by vegetative fragments (Kimbél 1982, Madsen et al. 1988). Localized spread is by root crowns and runners (Madsen et al. 1988, Madsen and Smith 1997). Although viable seeds are formed, they are not generally significant in the perennation or spread of the plant (Madsen and Boylen 1989, Hartleb et al. 1993).

The invasion process for nonindigenous species follows a progression from introduction, establishment, and colony formation stages. Each step of this process, and subsequent growth, is moderated by environmental factors affecting the outcome. The subsequent growth of the colony is affected by a broad suite of abiotic and biotic factors. The abundance of the invading plant can be described by a Gaussian relationship (Figure 1A). The curved solid line represents the upper boundary of abundance. Plant abundance also occurs below the line when limited by other environmental parameters limiting to growth, biotic activity, disturbance occurring to reduce abundance, or insufficient time elapsing to reach maximal levels. If the maximal level is of interest, then the best approach is to approximate an upper boundary to the plant abundance (Figure 1A, dashed line).

The goals of this study were to correlate limnological parameters to Eurasian watermilfoil dominance, and from these relationships to develop estimates predicting invasion success. This tool would then be used to allocate resources towards monitoring and managing lakes most likely to develop problem populations of Eurasian watermilfoil.

## MATERIALS AND METHODS

A literature review of lakes with Eurasian watermilfoil populations resulted in data for over 300 lakes from 30 publications and 14 unpublished sources that indicated both Eurasian watermilfoil dominance and relevant limnological data. Details of this data set are given elsewhere<sup>3</sup>. Data was obtained for lakes in Vermont, New York, Michigan, Wisconsin, Minnesota, Washington, Oregon, Alabama, Ontario, and British Columbia. Typically, only one year of data was obtained for each lake.

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<sup>2</sup>U.S. Geological Survey, Biological Resources, Gainesville, FL. August 1997. Worldwide Web Homepage: <http://nas.nfrcg.gov/dicots/>.

<sup>3</sup>Madsen, J. D. 1997. Predicting Eurasian Watermilfoil Invasion Success for Minnesota Lakes. Letter report, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS. In preparation.



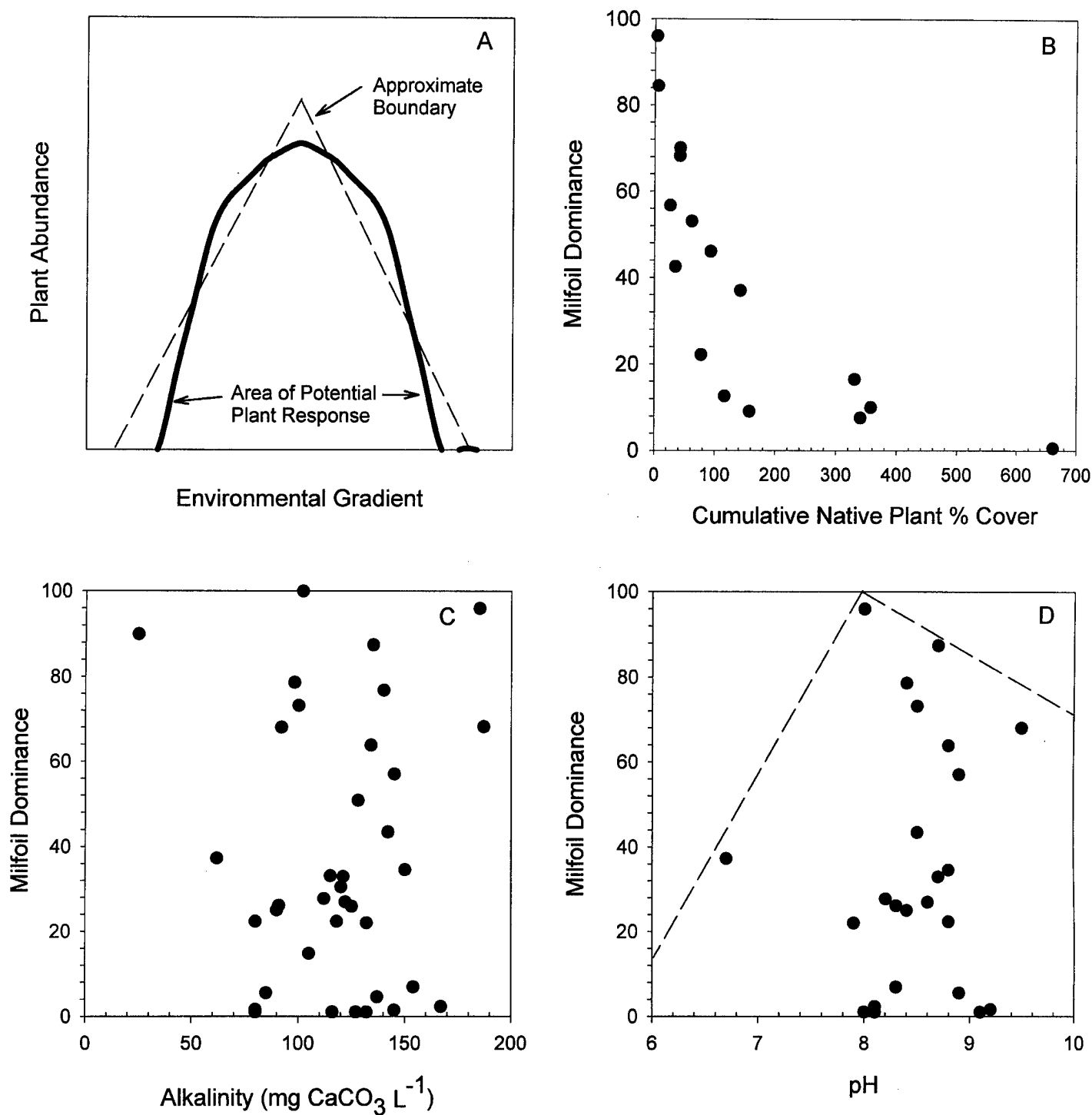


Figure 1. A) Diagram of a theoretical Gaussian distribution of plant abundance along an environmental gradient (solid line), and an approximated boundary (dashed line); B) Relationship between Eurasian watermilfoil dominance (as percent of littoral zone with Eurasian watermilfoil) and native plant cover in 17 lakes; C) Relationship between Eurasian watermilfoil dominance and alkalinity (mg  $\text{CaCO}_3 \text{ L}^{-1}$ ) in 39 lakes; D) Relationship between Eurasian watermilfoil dominance and pH in 25 lakes, with an approximate boundary indicated with a dashed line.

Plant abundance and distribution were measured in various ways and reported in different units. These diverse methods have been converted into a measure of Eurasian watermilfoil dominance, which is computed as the proportion, or percent, of the littoral zone in which Eurasian water-

milfoil was found. The necessity of restricting Eurasian watermilfoil data to this measure resulted in the discarding of some lakes and studies from consideration. A total of 102 lakes had sufficient data to calculate the estimate of Eurasian watermilfoil dominance. Plant community data included:

aquatic plant species presence and/or abundance, Eurasian watermilfoil biomass, Eurasian watermilfoil percent cover, native plant percent cover, Eurasian watermilfoil cover area (littoral zone), native plant cover area (littoral zone). Lake morphometry information for each lake included: maximum depth, average depth, area, littoral zone area, and shoreline development. Limnological data sought included: Secchi disk depth, light attenuation coefficient, alkalinity, total P, P loading rate, total N, N loading rate, Trophic State, Carlson's Trophic State Index (TSI, Carlson 1977), dissolved inorganic carbon and acid neutralizing capacity. Other information that was included: state, county, township, geoposition (latitude, longitude), glaciated vs. unglaciated, soils, soil erosion rate, sedimentation rate, and land use. For most variables, insufficient data were found to continue analysis. Of the 31 parameter groups investigated, data will be presented for seven: cumulative native plant cover (the sum of the cover of native plant species), Secchi Disk depth, alkalinity, pH, sediment sand content, water column total phosphorus, and Trophic State Index. Since not all lakes in the ensuing analysis had data for the above parameters available, the number of lakes per plot were not constant. No lakes were deleted as outliers.

## RESULTS AND DISCUSSION

Before discussing the relationship of Eurasian watermilfoil to the environment, one other relationship bears examination. The abundance of Eurasian watermilfoil was inversely related to cumulative native plant cover (Figure 1B). Lakes with more than 50% Eurasian watermilfoil dominance were found to have less than 60% cumulative native plant cover. Although this has been quantitatively documented in one instance for a given lake over time (Madsen et al. 1991) and reported as occurring in other systems (Aiken et al. 1979, Grace and Wetzel 1978, Smith and Barko 1990), this documents a relationship for many lakes over a range of Eurasian watermilfoil dominance.

Dissolved organic carbon or alkalinity has often been cited as a parameter associated with the success of Eurasian watermilfoil in lakes (Grace and Wetzel 1978, Smith and Barko 1990). In fact, the photosynthetic rate in Eurasian watermilfoil has been correlated to dissolved inorganic carbon for a group of Italian lakes (Adams et al. 1978). Nevertheless, the present study indicated abundant Eurasian watermilfoil across a broad range of alkalinity (Figure 1C). Other studies have also observed the occurrence of Eurasian watermilfoil across a broad range in alkalinity, but have not generally measured Eurasian watermilfoil abundance<sup>4</sup>. A similar plot of Eurasian watermilfoil dominance versus pH appears to give a relationship (Figure 1D), but pH is a highly variable parameter. Likewise, the low number of lakes at the low end of the pH spectrum severely limit the usefulness of this relationship. Eurasian watermilfoil is not typically found in abundance in either clearwater or brownwater acid lakes (Warrington 1985).

Light is often recognized as a parameter that controls the presence of submersed aquatic plants (Barko et al. 1986), but it is a poor predictive tool for Eurasian watermilfoil dominance relative to native plants due to its widespread effect on all plants. A plot of Eurasian watermilfoil dominance versus Secchi Disk depth, as a measure of lake transparency, indicates that Eurasian watermilfoil is abundant in some very low transparency lakes (Figure 2A).

Sediment fertility has also been evaluated related to the growth of Eurasian watermilfoil, as with other submersed macrophytes (Smith and Barko 1990). Growth limitation of Eurasian watermilfoil due to insufficient sediment nitrogen has been documented (Anderson and Kalff 1986). Unfortunately, few lakes are monitored for sediment nitrogen levels. One possible correlate is the percent composition of sand in sediment. Sandy sediments are known to be of low fertility (Barko et al. 1986). A plot of Eurasian watermilfoil dominance versus percent sand composition of sediments (Figure 2B) indicates a potential maximal limit which increases from 10% sand to 18% sand, possibly indicating the low growth potential of plants rooted in highly organic sediments. Above 18% sand, the upper limit of Eurasian watermilfoil dominance declines, which may be indicative of reduced fertility and growth rates. The upper limit of Eurasian watermilfoil dominance is still 80% when the sediment composition is essentially 100% sand. This plot demonstrates a very low potential to discriminate between high and low dominance of Eurasian watermilfoil and relies too heavily on only four points for its shape. One confounding factor in this instance is that groundwater often percolates through sandy sediments, which may replenish the concentrations of nutrients in these sediments (Loeb and Hackley 1988, Lodge et al. 1989).

Eurasian watermilfoil dominance exhibits possibly the most distinct and predictive relationship with total water column phosphorus (Figure 2C). The shape of this relationship most closely approximates that expected in a theoretical Gaussian relationship, being broad at the base and narrow at the top. Eurasian watermilfoil dominance increases sharply as water column phosphorus increases from oligotrophic ( $<10 \mu\text{g L}^{-1}$ ) through mesotrophic ( $<30 \mu\text{g L}^{-1}$ ) concentrations, and decreases above  $50 \mu\text{g L}^{-1}$ , in what is considered moderately eutrophic lakes (Wetzel 1983, Carlson 1977). Eurasian watermilfoil, however, is probably not responding directly to water column phosphorus. Experimental studies have indicated that Eurasian watermilfoil is generally limited by nitrogen availability (Barko 1983, Anderson and Kalff 1986), and that phosphorus is taken up from the sediment rather than water column (Carignan and Kalff 1979, 1980, Barko and Smart 1981). Total water column phosphorus may be a correlative variable for several environmental factors, including sedimentation (which initially stimulates plant growth) and phytoplankton abundance, which would shade Eurasian watermilfoil (Jones et al. 1983).

The plot of Eurasian watermilfoil dominance versus Carlson's Trophic State Index (TSI, Carlson 1977, Figure 2D) indicates a narrower margin of abundant Eurasian watermilfoil than might be expected. Eurasian watermilfoil was found in lakes ranging from 35 (transitional oligotrophic) to 70 (moderately eutrophic). Mesotrophic lakes are typically

<sup>4</sup>Crow, G. E. and C. B. Hellquist. 1983. Aquatic Vascular Plants of New England: Part 6. Trapaceae, Haloragaceae, Hippuridaceae. New Hampshire Agricultural Experiment Station Bulletin 524, University of New Hampshire, Durham, NH. 26 p.

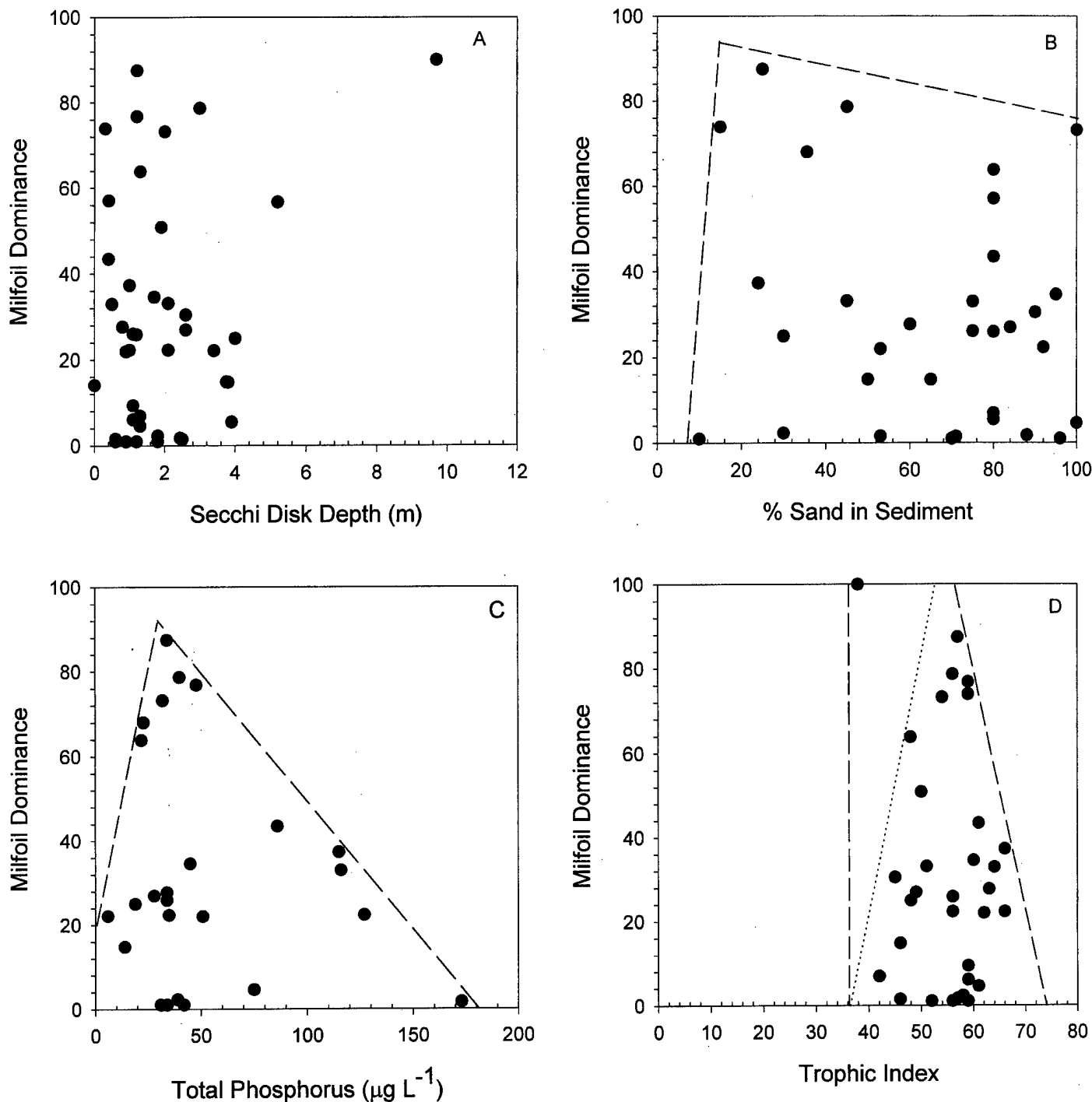


Figure 2. A) Relationship between Eurasian watermilfoil dominance (as percent of littoral zone with Eurasian watermilfoil) and Secchi Disk depth (m) for 42 lakes; B) Relationship between Eurasian watermilfoil dominance and percent sand content of sediment for 33 lakes, with an approximate boundary indicated with a dashed line; C) Relationship between Eurasian watermilfoil dominance and water column total phosphorus ( $\mu\text{g L}^{-1}$ ) for 25 lakes, with an approximate boundary indicated with a dashed line; and D) Relationship between Eurasian watermilfoil dominance and Carlson's trophic index for 34 lakes, with an approximate boundary indicated with a dashed line, and an additional approximate lower boundary disregarding one point indicated with a dotted line.

between 40 to 50 TSI (Cooke et al. 1986). This analysis corroborates observations that Eurasian watermilfoil actually appears most abundant in mesotrophic lakes and moderately eutrophic lakes (Smith and Barko 1990). If the abundant

Eurasian watermilfoil lake at 35 TSI is excluded, then the remaining relationship indicates a sharp increase in abundance from 35 TSI to 55 TSI, and a decline from 55 TSI to 75 TSI.

In a preliminary attempt to identify factors that might predict the eventual success of Eurasian watermilfoil in infested lakes, total water column phosphorus and Carlson's TSI were identified as potential indicators of lakes at risk. From this analysis, lakes with a TP of 20-60  $\mu\text{g L}^{-1}$  or a Carlson's TSI of 45-65 were most at risk of dominance by Eurasian watermilfoil. Using this type of tool, monitoring and management resources might be allocated to those lakes most likely to develop substantial nuisance growths of Eurasian watermilfoil, with the accompanying impacts to both human use and the lake ecosystem.

## ACKNOWLEDGMENTS

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# The Aquatic Macrophyte Seed Bank in Lake Onalaska, Wisconsin

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## ABSTRACT

Submersed aquatic vegetation, dominated by *Vallisneria americana* Michx., declined dramatically in Lake Onalaska (Navigation Pool 7, on the Upper Mississippi River) following drought conditions in the late 1980s. Coinciding with the decline were marked increases in the abundance of *Myriophyllum spicatum* L., particularly in areas vacated by *V. americana*. Recent evidence indicates that much of the lake has remained unvegetated, but that since 1994, beds of *V. americana* have made a partial recovery. While the production of vegetative propagules may largely account for increases in populations of both species, the extent to which seed production may contribute to their expansion in the lake is unknown. To assess the germination potential and distribution of the aquatic macrophyte seed bank in Lake Onalaska, sediment cores (5 cm deep) were collected from 74 sampling sites in July 1996. Seedling emergence from sediments was observed in an environmental growth chamber operated at 25 C and a 14-hr photoperiod over a period of eight weeks. Fifteen species of aquatic macrophytes germinated in sediments from 55 sites. *V. americana* seedlings emerged from sediments from 36 sites throughout the lake, but were most prevalent in sediments collected within or downstream (within 250 m) of established *V. americana* beds. Seedlings of *M. spicatum* emerged from only two collected sediments that had supported this species in protected areas. These findings suggest that seed production may play a greater role in the dispersal of *V. americana* than *M. spicatum*, and further emphasize basic differences in their survival strategies, particularly in flowing water systems.

**Key words:** Aquatic plants, *Vallisneria americana*, *Myriophyllum spicatum*, sexual reproduction, germination.

## INTRODUCTION

Sexual and asexual reproduction are the two fundamental means by which both terrestrial and aquatic macrophytes propagate (Sculthorpe 1967, Salisbury and Ross 1985). Because sexual reproduction provides genetic variation, it is considered the more advantageous mode in highly dynamic or heterogeneous environments, such as those experienced mainly by terrestrial macrophytes. Conversely, asexual reproduction maintains genetic uniformity through cloning, and

appears most effective in species adapted to relatively stable surroundings (Hartog 1970, Williams 1975, Grant 1981, Grace 1993, Philbrick and Les 1996). Despite differences that exist among aquatic systems, overall they tend to demonstrate greater chemical and thermal stability than terrestrial systems, and can function as temporary buffers against catastrophic events, e.g., dramatic shifts in temperature, flooding, fire, and wind storms (Sculthorpe 1967, Wetzel 1975, White et al. 1992, Philbrick and Les 1996). From an evolutionary standpoint, it is not surprising that asexual reproduction has become the dominant means of aquatic macrophyte population expansion. Yet, the abilities to flower and set seed have generally been retained even in the most clonal species of aquatic macrophytes (Sculthorpe 1967, Spencer and Bowes 1993).

The emphasis on asexual reproduction in aquatic macrophytes is reflected in their production of a variety of specialized vegetative propagules. Except for certain aquatic annual species in which reproduction is exclusively sexual, aquatic macrophytes are predominantly perennial (Sculthorpe 1967, Philbrick and Les 1996), producing such propagules as tubers, turions, corms, and stolons as a means of overwintering (Sculthorpe 1967, Hutchinson 1975, Grace 1993, van Vierssen 1993). In temperate species, these propagules are typically formed during short photoperiods of autumn; they remain dormant in the sediment throughout the winter, and germinate with warming temperatures of spring (Sculthorpe 1967, Hutchinson 1975, Grace 1993, van Vierssen 1993). During the growing season, lateral expansion of a population occurs vegetatively through rhizomes and stolons that root and form young plantlets at the nodes (Sculthorpe 1967, Grace 1993).

One of the most important mechanisms of long-distance dispersal is through stem fragmentation. However, the propensity for fragmentation varies substantially among macrophyte species with different growth forms. For example, the exotic species *M. spicatum* exhibits an elongated growth habit which concentrates much of the stem mass in the water column and especially on the water surface. Its long delicate stems are easily broken during senescence and can be widely distributed by water currents and wave action (Madsen et al. 1988). Unlike *M. spicatum*, germinable portions of the stem in the native species *V. americana* (i.e., stolons, rhizomes, tubers) are buried or anchored in sediment, and are less likely than stems of *M. spicatum* to be swept away by water movements. Notably, however, *V. americana* is well-adapted for water-mediated pollination (Korschgen and Green 1988) and has been observed to produce large numbers of fruits in established beds in the field (Korschgen and Green 1988; S. J. Rogers<sup>2</sup>, pers. observ.). Thus, in sexually viable populations of this species, fruit and seed production may be the most effective mechanism of dispersal and colonization.

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Although seed banks of terrestrial and wetland species have been extensively studied (e.g., Major and Pyott 1966, Wein and Bliss 1973, Huiskes et al. 1995, Leck and Simpson 1995, Henry and Armoros 1996, van der Valk and Rosburg 1997, Westcott et al. 1997), few studies have examined the seed banks of submersed aquatic macrophytes (Haag 1983, Madsen et al. 1993, Kimber et al. 1995). This may be due in part to the fact that seeds of submersed macrophytes are quite small and are rarely observed to grow into mature plants in nature. Consequently, their role in population dynamics is largely overshadowed by that of vegetative propagules and often presumed to be minor. Yet, for certain species, as in *V. americana*, seed production may not only be the primary means of propagule dissemination, but an important source of propagule longevity. Despite the small size and little carbohydrate reserve in submersed macrophyte seeds, they appear to withstand adverse conditions and can remain viable for several years (Sculthorpe 1967). With these considerations, the availability of a viable seed bank could be crucial to the recovery of an aquatic plant community following a severe or prolonged habitat disturbance (e.g., drought or drawdown).

The need to investigate the aquatic macrophyte seed bank in Lake Onalaska, Wisconsin became evident following a widespread three-year drought that peaked in 1988. Over 1214 ha of submersed aquatic vegetation dominated by *V. americana* dramatically declined, and by 1990, less than 121 ha were estimated to remain (Rogers 1994, Kimber et al. 1995, Rogers et al. 1995). The loss of this species prompted concern over increasing populations of *M. spicatum*, a potentially nuisance species that had begun to colonize areas vacated by *V. americana*. The restoration of *V. americana* was favored because of its highly valued contributions to lake ecology (Rogers et al. 1995). Established stands of *V. americana* help to improve water quality by stabilizing sediments and filtering out suspended particles. Also, the rootstocks and winter buds of this species are an important food resource for canvasback ducks and other waterfowl (Korschgen and Green 1988, Korschgen et al. 1988).

In this article, we present results of an investigation of the germination potential and distribution of the seed bank of aquatic macrophytes in Lake Onalaska. The study was conducted jointly by the US Geological Survey (USGS), Onalaska, Wisconsin, and the Environmental Laboratory of the US Army Engineer Waterways Experiment Station (WES), Vicksburg, Mississippi. The specific objectives of the investigation were to: 1) examine seedbank diversity by identifying seedlings that emerge from sediments from different sampling locations, 2) compare densities of germinable seeds at different sediment depths, and 3) determine frequencies of species that germinate in the laboratory for comparison with frequencies of species observed in the field. The results are intended to provide insight into the role of seed banks in the recurrence and spread of aquatic macrophyte populations, with particular reference to *V. americana* and *M. spicatum*.

## STUDY AREA

Lake Onalaska is a large (2,835-ha), irregularly-shaped impoundment located in the backwaters of the Upper Mississippi River System (UMRS, Figure 1). The lake is situated in

the lower half of Navigation Pool 7, the region in the UMRS extending from Lock and Dam 6 near Trempealeau, Wisconsin downstream to Lock and Dam 7 near Dresback, Minnesota. Pool 7 was inundated by the US Army Corps of Engineers in 1937 and is operated to help maintain adequate depths for navigation in the river channel during periods of low-water flow (Chen and Simons 1986, Hendrickson and Hasse 1994<sup>3</sup>). West of the Mississippi River, Lake Onalaska is bordered by hills, bluffs, and scattered farmlands, and east of the river, by a narrow strip of marshlands and the city of Onalaska, Wisconsin (Korschgen et al. 1987; S. J. Rogers<sup>2</sup>, pers. observ.). At normal full-pool elevation (195 m based on National Geodetic Vertical Datum, 1912 adjustment; NGVD), water depths in the lake range up to about 2.5 m, with a mean depth of 1.4 m (Korschgen et al. 1988, Hendrickson and Hasse 1994<sup>3</sup>, Kimber et al. 1995). The Black River, the primary tributary, enters the lake directly from the north and east, and indirectly through a network of distributaries joined to the Mississippi River. Inflows from the Mississippi are received through seven secondary channels that cut through a long chain of barrier islands. The largest of these channels, Sommer's Chute, delivers up to 80% of the total input from the Mississippi River (Pavlou et al. 1982, Hendrickson and Hasse 1994<sup>3</sup>). The outlet structure for Pool 7 has both surface and below-surface discharge ports; flood flows (in excess of 82,000 cfs) are allowed to pass with minimal blockage through vertical slide gates that can be lifted completely out of the water and an earthen fixed-crest spillway that can be overtopped (Sparks 1995). Lake levels are typically stable except during major floods because of the close proximity of the lake to Lock and Dam 7 and the associated spillway structure (Korschgen et al. 1988). Peak discharge rates (above 80,000 cfs) from Pool 7 usually occur during snowmelts of spring and decrease to an overall seasonal minimum (typically < 20,000 cfs) by late summer or fall (St. Paul District 1971<sup>4</sup>, Hendrickson and Hasse 1994<sup>3</sup>). As rates of inflow to Pool 7 change, the water surface elevation at the dam is adjusted to 639 ft (195 m) NGVD, while elevations are allowed to vary at all other points in the pool (St. Paul District 1971<sup>4</sup>).

Lake Onalaska historically has been a productive lake, supporting an abundance of wildlife, sport fish and aquatic vegetation (Fleener 1975<sup>5</sup>, Holzer and Ironside 1977<sup>6</sup>, Rach and Meyer 1982, Mohlenbrock 1983<sup>7</sup>, Korschgen et al. 1987 and 1988, Rogers 1994). An important feature of the lake is

<sup>3</sup>Hendrickson, J. S. and F. R. Haase. 1994. Dynamic conditions in the Black River Delta/Lake Onalaska area, Pool 7, Upper Mississippi River, 1980-81 and 1991-92. US Army Corps of Engineers, St. Paul District, St. Paul, MN 55101-1638.

<sup>4</sup>St. Paul District. 1971. Mississippi River Nine Foot Channel Navigation Project, Appendix 7, Lock and Dam No. 7, La Crosse, Minnesota. US Army Corps of Engineers, St. Paul, MN.

<sup>5</sup>Fleener, G. G. 1975. The 1972-1973 sport fishery survey of the Upper Mississippi River. Upper Mississippi River Conservation Committee, Rock Island, IL.

<sup>6</sup>Holzer, J. A. and S. J. Ironside. 1977. Basic lake inventory of Lake Onalaska, La Crosse, Wisconsin. Wisconsin Department of Natural Resources, La Crosse, WI.

<sup>7</sup>Mohlenbrock, R. H. 1983. Annotated bibliography of the aquatic macrophytes of the Upper Mississippi River covering the area from Cairo, Illinois to St. Paul, Minnesota. US Fish and Wildlife Service, Rock Island, IL.

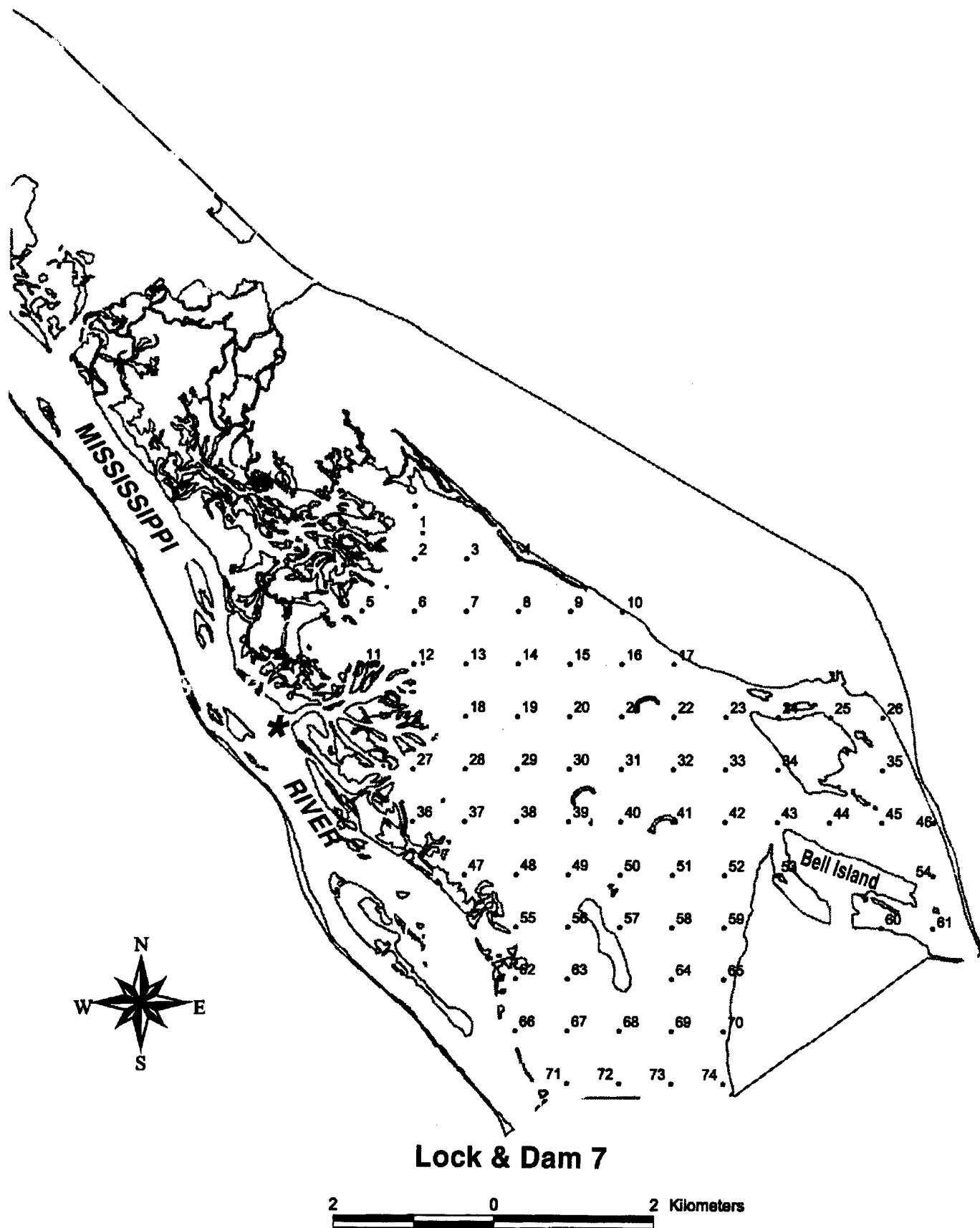


Figure 1. Lake Onalaska (Pool 7 of the Upper Mississippi River), Wisconsin with sampling site locations. Asterisk (\*) indicates inlet of Sommer's Chute.

that it is managed as a wildlife refuge that serves as the staging ground for up to 75% of the world's canvasback duck population (Korschgen et al. 1988). The lake provides an extensive open water habitat with beds of aquatic macrophytes occurring mainly near the lake margins and island perimeters. Surveys of aquatic vegetation over a period from 1990 to 1996 indicate that at least 14 species of submersed aquatic macrophytes occurred in the lake (i.e., *V. americana*, *Heteranthera dubia* (Jacq.) MacM., *Potamogeton crispus* L., *Ceratophyllum demersum* L., *Najas flexilis* (Willd.) Rostk., *P. richardsonii* (Benn.) Rydb., *P. pectinatus* L., *P. foliosus* Raf., *Chara* sp., *Zannichellia palustris* L., *P. zosteriformis* Fernald, *Elodea canadensis* Rich. in Michx., *M. spicatum*, and *P. nodosus* Poiret) and that *V. americana* and *M. spicatum* were among the most common (Rogers 1994, USGS unpubl. data).

## MATERIALS AND METHODS

In July 1996, sediments were collected by the USGS from 74 sampling sites (Figure 1). The positions of the sites were generated using a systematic sampling grid and located in the field with a Precision Lightweight GPS (Global Positioning System) unit. At each location, five sediment cores were obtained using a 10-cm diameter coring device, and sediment within the upper 5 cm of each core was retrieved. As the sediments were collected, they were divided equally into two 2.5-cm-deep sections. Shallow sections were selected to allow comparisons between sediment depths from which seedlings were most likely to emerge (cf. Hartleb et al. 1993). The upper sections of sediment were composited as were the lower sections to reduce sampling error associated with seed clumping (Bigwood and Inouye 1988). Afterwards, the composites were sifted by hand to remove unwanted debris (e.g., rocks and twigs) and any asexual propagules (e.g., fragments, tubers, and turions). When sediment collection was completed, there were 148 ( $\approx$  980-ml) composites: 74 each from the upper (0 to 2.5 cm) and lower (2.5 to 5.0 cm) sediment sections. These composites were placed separately in gallon-sized Zip-loc bags, and shipped in coolers to WES within five days.

At WES, the composites were established in plastic planting containers and observed under controlled environmental conditions for seedling emergence. The containers (20 cm  $\times$  20 cm  $\times$  8 cm deep) were each filled with 600 ml of a single well-mixed composite spread evenly to depth of 1.5 cm. Standard culture solution (described below) was then poured into each flat, producing a 6-cm-deep water column overlaying the sediment. A clear Plexiglas lid was placed on top of each container to help reduce evaporative losses. Prepared containers were aligned on benches in an environmental growth chamber operated to maintain a 14-hr photoperiod, at 350  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  PAR (photosynthetically active radiation), and a constant temperature of 25 C. Assessments of seedling emergence were based on direct counts and recorded at weekly intervals over a period of eight weeks. A detailed description of the growth chamber and ancillary equipment is presented by Barko and Smart (1980 and 1981).

The low alkalinity culture solution that was used in the study was prepared according to Smart and Barko (1985). This solution, formulated with reagent-grade salts and deionized-distilled water, provides major cations ( $\text{Na}^+ = 16.0$ ,  $\text{K}^+ =$

$6.0$ ,  $\text{Ca}^{+2} = 25.0$ , and  $\text{Mg}^{+2} = 6.8 \text{ mg}\cdot\text{L}^{-1}$ ) and anions ( $\text{Cl}^- = 44.2$ ,  $\text{HCO}_3^- = 51.8$ , and  $\text{SO}_4^{+2} = 26.9 \text{ mg}\cdot\text{L}^{-1}$ ) but lacks N and P, specifically omitted to reduce algal growth. Upon preparation, the solution had a pH of 7.9 and an electrical conductivity of 278  $\mu\text{S}\cdot\text{cm}^{-1}$  at 25 C. A single air line per container provided filtered-humidified air to enhance mixing and air/water  $\text{CO}_2$  exchange. The solution was replaced at least twice weekly to minimize cloudiness from transplanting and invertebrate activity.

Approximately two weeks after emergence, individual seedlings were transplanted using procedures similar to those for aquatic plant cultures (Smart and Barko 1985). The tiny seedlings were gently uprooted from their original culture flats and planted singly in sediment contained in 300-ml plastic cups. The sediment that was used was collected from Brown's Lake, WES and was amended with nitrogen by adding 0.8 g  $\text{NH}_4\text{Cl}\cdot\text{L}^{-1}$  wet sediment. Physical and chemical characteristics of this substrate are presented by McFarland and Barko (1987) and McFarland et al. (1992). When planted, a thin layer of washed builder's sand was placed over the sediment surface and the cups were submersed in culture solution in 200-L fiberglass tanks. The seedlings were grown under chamber conditions for at least four weeks after transplanting to allow ample time for plant development needed for species identification. The identifications here are based on descriptions of plant morphology provided by Fernald (1970), Fassett (1975), and Godfrey and Wooten (1979 and 1981).

Data from this study were analyzed using analysis of variance (ANOVA) and post-ANOVA procedures of the Statistical Analysis System (SAS Institute, Inc. 1991). The general linear model (GLM) procedure was applied in cases involving unequal sample sizes. Tests of normality were performed using the Shapiro-Wilk statistic; homogeneity of variance was evaluated using the Levene's test. Separation of means was accomplished as appropriate using Duncan's multiple range test or Fisher's LSD (Least Significant Difference) Test. Hereafter, statements of statistical significance without precise indication of probability level refer to  $P \leq 5\%$ .

## RESULTS AND DISCUSSION

A total of 15 aquatic plant species, representing a variety of growth forms, emerged from sediments from 55 of the 74 sampling sites (Table 1). Among these, ten species were submersed (i.e., *V. americana*, *N. flexilis*, *H. dubia*, *P. foliosus*, *P. crispus*, *M. spicatum*, *P. richardsonii*, *C. demersum*, *Chara* sp. and *Nitella* sp.), three were emergent (i.e., *Sagittaria latifolia* Willd., *Lindernia dubia* (L.) Pennell, and *Sparganium eurycarpum* Engelm.), and two were free-floating aquatic plants (i.e., *Lemna minor* L. and *Spirodela polyrrhiza* (L.) Schleiden). The emergence of seedlings continued over the entire eight weeks of the study, but there was little increase in the number of seedlings beyond six weeks. Seedlings of *S. latifolia*, *P. crispus*, *H. dubia*, and *N. flexilis* began appearing within the first week of study initiation, while *V. americana* and *M. spicatum* seedlings took nearly two weeks or more to emerge. The most widespread species was *V. americana*, occurring in sediments from 36 sites. *C. demersum* was the rarest species, occurring in samples from only one site. The number of seedlings (or sporelings) of each species did not vary significantly



TABLE 1. AQUATIC PLANT SPECIES GERMINATED IN SEDIMENTS FROM LAKE ONALASKA, WISCONSIN.

Species	No. of Sites	Estimated Density
<i>Vallisneria americana</i> Michx.	36	36.2 (4.6)
<i>Sagittaria latifolia</i> Willd.	25	56.8 (13.4)
<i>Lindernia dubia</i> (L.) Pennell	22	54.4 (11.6)
<i>Najas flexilis</i> (Willd.) Rostk.	11	17.9 (3.6)
<i>Sparganium eurycarpum</i> Engelm.	10	30.2 (7.9)
<i>Chara</i> sp.	10	40.8 (14.1)
<i>Heteranthera dubia</i> (Jacq.) MacM.	9	20.4 (4.4)
<i>Potamogeton foliosus</i> Raf.	6	32.9 (12.6)
<i>Nitella</i> sp.	5	28.9 (9.7)
<i>Potamogeton crispus</i> L.	5	21.0 (3.2)
<i>Lemna minor</i> L.	4	44.0 (12.2)
<i>Spirodela polyrrhiza</i> (L.) Schleiden	4	26.3 (7.6)
<i>Myriophyllum spicatum</i> L.	2	19.7 (6.6)
<i>Potamogeton richardsonii</i> (Benn.) Rybd.	2	19.7 (6.6)
<i>Ceratophyllum demersum</i> L.	1	13.1 (0.0)

\*Density = no. of seedlings (or sporelings)/m<sup>2</sup>. Values are means ( $\pm 1$  std. err.), where  $n$  = no. of sites.

between sediment depths ( $P > 0.05$ ); therefore, the results presented here are based on calculations where data for upper and lower sediment sections were combined.

Figure 2 presents a synopsis of seedling emergence from sediments from the 74 sampling sites. Within each subfigure, the sites are categorized according to position within a 250-m radius of the center of an established plant bed. This information was derived using point data provided by the USGS indicating the positions of aquatic plant beds in Lake Onalaska in 1996. According to those determinations, 31 sampling sites were located in open areas (i.e., outside the set 250-m radius), and of the remaining 43 sampling sites, 16 were situated in the center of a plant bed, 13 were generally south, and 14 generally north of a vegetated area (Figure 2A). The percentage of sites that showed germination was greatest in sites located either in the middle or downstream (south) of established vegetation (Figure 2B); those same sites also demonstrated significantly higher densities of seedlings ( $P < 0.05$ ) and numbers of species ( $P < 0.05$ ) than sites in the other two categories (Figures 2C and 2D). A similar trend was apparent when the distribution of *V. americana* seedlings was considered (not shown). Densities of *V. americana* seedlings taken from the center or south of a *V. americana* bed (31.4 and 40.7 seedlings/m<sup>2</sup>, respectively) were significantly greater than those taken from open areas or upstream of a *V. americana* bed (14.4 and 6.6/m<sup>2</sup> for sites, respectively). Maps of water movements in Lake Onalaska show a prevailing southward flow pattern in the lake (USGS unpubl. data), coinciding with the observed southward drift and deposition of seeds relative to plant bed location.

Although 30 sampling sites were located in the vicinity of *M. spicatum* beds, sediments from only two sites showed emergence of *M. spicatum* (Table 1). Interestingly, the two sites (60 and 61) occurred in protected areas that had supported stands of this species near Bell Island (Figure 1). On-site observations by the USGS indicated a typical absence of flowering and seed set in *M. spicatum* since at least 1991 (S. J. Rogers<sup>2</sup>, pers. observ.). Restricted sexual reproduction appears to have had little impact on *M. spicatum*'s occurrence as it was relatively common in Lake Onalaska according to

1996 surveys (USGS unpubl. data). The scarcity of its seedlings in the present study, along with previous USGS data, suggest that: 1) seed production and dispersal may be of minor importance to the spread of *M. spicatum* in Lake Onalaska, and that 2) the capacity of *M. spicatum* to develop a substantial seed bank in the lake appears minimal compared to that of *V. americana*.

Numerous studies have reported moderate to high rates of germination in seeds of *V. americana* (Muenscher 1936, Kimber et al. 1995) and *M. spicatum* (Patten 1955, Coble and Vance 1987, Madsen and Boylen 1988<sup>8</sup>, Hartleb et al. 1993). However, few have demonstrated the growth of seedlings of these species under favorable conditions after emergence. Although early attempts to grow seedlings of *M. spicatum* in the laboratory failed to produce healthy plants (Anonymous 1981<sup>9</sup>), subsequent efforts of McDougall (1983<sup>10</sup>) suggested that *M. spicatum* seedlings could achieve a mature size (Hartleb et al. 1993). Additionally, Titus and Hoover (1991) reported that seedlings of *V. americana* transplanted in Otsego Lake accrued a substantial mean of 1.9 g dry mass and 6.3 rosettes ( $n = 16$ ) in less than 16 weeks. In the present study, seedlings of most species grew better than expected and had to be transferred (singly) from cups into larger (3-L) sediment containers. Twelve weeks after the initial transplanting, seedlings of *M. spicatum* had reached 188.1 ( $\pm 22.3$ ) cm in height (mean  $\pm 1$  std. err.,  $n = 3$ ) and 10.7 ( $\pm 1.2$ ) g total dry mass. Over approximately the same growth period, seedlings of *V. americana* reached a height of 91.9 ( $\pm 6.8$ ) cm and a total dry mass of 17.3  $\pm 1.3$  g ( $n = 6$ ). These were not the only species to demonstrate such capacities. Seedlings of *P. crispus*, *P. foliosus*, *P. richardsonii*, *S. latifolia*, *L. dubia*, and *H. dubia* all demonstrated substantial growth subsequent to transplanting.

Given the potential for submersed macrophyte seedlings to accrue significant amounts of biomass, it is important to understand mechanisms that affect their establishment and survival. A variety of environmental factors such as water movements, sedimentation, competition, and grazing undoubtedly limit seedling growth *in situ*. Moreover, the environmental tolerances of newly-emerged seedlings are probably narrower than those of established plants, and in nature, their survival may be severely restricted by suboptimal conditions that might exist (Smith et al. 1991). Nevertheless, the fact that a forbidding array of limitations may be imposed on seedlings *in situ* does not eliminate altogether the prospects for successful seedling establishment. One of the clearest examples is apparent in Lake Onalaska where over the past four years submersed annuals including *P. folio-*

<sup>8</sup>Madsen, J. D. and C. W. Boylen. 1988. Seed ecology of Eurasian water-milfoil (*Myriophyllum spicatum* L.). Rensselaer Fresh Water Institute Report 88-7. Rensselaer Polytechnic Institute, Troy, NY.

<sup>9</sup>Anonymous. 1981. A Summary of Biological Research on Eurasian water milfoil in British Columbia. Information Bulletin. British Columbia Ministries of Environment, Aquatic Studies Branch.

<sup>10</sup>McDougall, I. A. 1983. A study of the germination potential in *Myriophyllum spicatum* L. seeds. Studies on Aquatic Macrophytes Part XXIV. Province of British Columbia Ministry of Environment, Water Management Branch, Victoria.

<sup>11</sup>Korschgen, C. E. US Geological Survey, Upper Mississippi River Science Center, P.O. Box 818, La Crosse, WI 54602-0818.

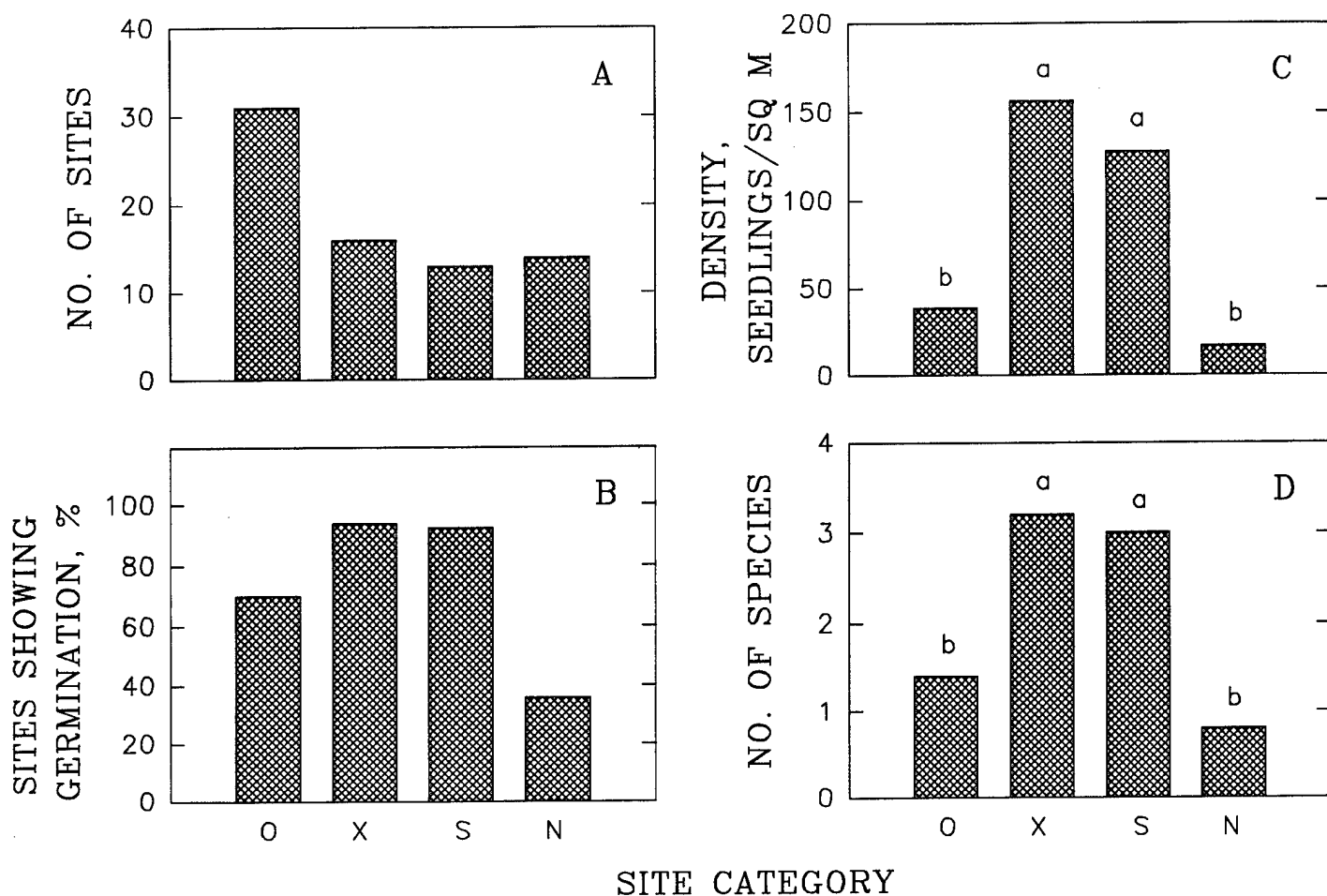


Figure 2. Number of sites (A), percentage of sites showing germination (B), seedling density (C), and number of species (D) in different site categories ('O' indicates sites in open areas; 'X,' sites in the center of a plant bed; 'S,' sites south or downstream of a plant bed; 'N,' sites north or upstream of a plant bed). Within subfigures C and D, means sharing the same letter (in lower case) do not differ at the 5% level of significance according to Fisher's Least Significant Difference Test.

*sus*, *N. flexilis*, and *H. dubia* have been increasing in abundance (USGS unpubl. data). Furthermore, young plants of *V. americana* with seed coats still attached have been observed growing in slow-flow areas of Lake Onalaska (Korschgen and Green 1988, Kimber et al. 1995; S. J. Rogers<sup>2</sup>, pers. observ., C. E. Korschgen<sup>11</sup>, pers. comm.). Kimber et al. (1995) also speculated that seedlings were the source of *V. americana* beds where they had been absent for five years or more. These observations and ours concerning the potential for seedling growth, suggest that the role of seed banks in vegetation recovery may be greater than previously surmised. Further studies of environmental influences on the growth of submersed macrophyte seedlings would be useful in assessing relative production potential of seedlings under site-specific conditions.

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# Phenological Studies of Carbohydrate Allocation in Hydrilla

CHETTA S. OWENS<sup>1</sup> AND JOHN D. MADSEN<sup>2</sup>

## ABSTRACT

Hydrilla (*Hydrilla verticillata* (L.f.) Royle), a nonindigenous submersed aquatic plant, was first introduced into the United States in the 1960's. Hydrilla exhibits aggressive growth, forming dense canopies of biomass at the surface of the invaded aquatic systems, affecting fisheries, water quality, transportation and recreational usage. Studies of the phenological seasonal cycles of invasive plants indicate optimal timing to apply management techniques. Biomass and total nonstructural carbohydrate (TNC) allocation of dioecious hydrilla were studied in outdoor ponds in Texas between January 1994 through July 1995. Biomass increased from May through September, growing from overwintering shoots and root crowns, not tubers. Tuber germination occurred in August. Tuber and turion production occurred from October through April. A carbohydrate storage minimum was observed in late July for 1994 and June for 1995, with storage generally split between stolon (7% TNC), root crown (10% TNC) and lower stem (16% TNC). Tubers and turions ranged from 58 to 68% TNC. These studies provide more insight into the timing of major allocation shifts in the hydrilla seasonal growth cycle.

**Key words:** *Hydrilla verticillata*, total nonstructural carbohydrates, Hydrocharitaceae, dioecious hydrilla.

## INTRODUCTION

Most native aquatic plants enhance their ecosystems. Some of the derived benefits include fish and waterfowl habitat, sediment stabilization and improved water quality (Madsen 1997a). However, many introduced species, including hydrilla, can have negative impacts. Invaded aquatic ecosystem are impacted by increased biomass and dense canopy production which affects water quality, especially dissolved oxygen. Further, dense canopy production shade out native vegetation, thereby leading to a loss of native diversity.

Hydrilla is native to Southeast Asia and Australia. The first discovery of hydrilla in the United States was in the state of Florida during the 1960's (Pieterse 1981). Two distinct biotypes (monoecious and dioecious) exist in the United States (Spencer and Anderson 1986). Monoecious hydrilla has both staminate and pistillate floral components on the same plant while dioecious biotypes produce staminate and pistil-

late on separate plants. Currently, within the United States, dioecious hydrilla is pistillate producing only; therefore, indicating that no seeds are produced. Dioecious hydrilla is found throughout the southeastern United States and as far north as Connecticut (Les et al. 1997).

Phenology is the study of the seasonal cycle of plants and animals. Previous plant phenology research has successfully demonstrated optimal timing for control of cattails<sup>3</sup>. The utility of herbicide application timing to carbohydrate depletion to improve weed management has also been demonstrated for the terrestrial weed quackgrass (Schirman and Buchholtz 1966). At the Lewisville Aquatic Ecosystem Research Facility (LAERF), phenology studies have been conducted on waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) (Luu and Getsinger 1990) and Eurasian watermilfoil (*Myriophyllum spicatum* L.) (Madsen 1997b).

Many aquatic plants have specific organs for storage of carbohydrates, such as tubers in sago pondweed (*Potamogeton pectinatus* L.), turions in curly-leaf pondweed (*P. crispus* L.) and stembases in waterhyacinth (Madsen 1991). Hydrilla has a number of different storage organs for carbohydrates, including tubers, turions, stolons and root crowns. Upper shoots perform photosynthesis, exporting carbohydrates to these storage organs for use during periods of overwintering or environmental stress. In the spring, these stored carbohydrates provide energy to the plant for regrowth. The goal of this study was to document seasonal changes in the storage of total nonstructural carbohydrates (TNC), expressed as percent TNC of dry weight and observe low points in carbohydrate storage which might be exploited for management of this species.

## METHODS

This study was conducted at the LAERF, in Lewisville, Texas (latitude 33°04'45"N, longitude 96°57'30"W), from January 1994 through July 1995. Two experimental ponds (0.3 ha) were utilized, with an average depth of 1.0 m and a maximum depth of 1.5 m. Daily water temperature were continuously monitored using an Omnidata Easy Logger™ Field Data Recording System adjacent to the hydrilla research ponds. Missing temperature data was obtained from the NOAA (National Oceanic and Atmospheric Administration) monthly summary for the Dallas-Ft. Worth Regional Airport<sup>4</sup>.

From January 1994 through July 1995, 12 biomass samples were collected monthly from each of the two ponds, using a

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<sup>3</sup>Linde, A. F., T. Janisch and D. Smith. 1976. Cattail-The significance of its growth, phenology and carbohydrate storage to its control and management. Tech. Bull. 94, WI Dept. Nat. Resourc., Madison, WI. 27 p.

0.1 m<sup>2</sup> quadrat. Biomass samples were separated into upper and lower shoots, root crowns, stolons, tubers and turions. Inflorescences, root crowns, tubers and turions were counted. All samples were dried at 55 C in a Blue M forced air oven (General Signal, Atlanta, GA) for a minimum of 48 hours, then weighed. After obtaining a dry weight, samples were finely ground using a Cyclone Sampling Mill (UDY Corp, Ft. Collins, CO), for carbohydrate analysis.

Plant samples were analyzed for TNC using a modified procedure by Swank et al. (1982). Total nonstructural carbohydrate (starch, hydrolyzable sugars, reducing sugars) extracts were incubated at 55C for 15 minutes with one unit of amyloglucosidase (Sigma A-3042) per milliliter of completely hydrolyzed starch before assaying for reducing sugars (Nelson 1944, Madsen 1997b).

Additional core tuber samples (40) were collected monthly from January 1994 through July 1995 using a Wildco sediment sampler (Saginaw, MI; model # 2424-L15) with a 4.5 cm wide cylinder. Samples were washed, tubers counted and then processed as above to obtain dry weight and TNC content.

## RESULTS AND DISCUSSION

Hydrilla exhibited an aggressive growth strategy as water temperatures increased. This is evident in the expansion in root crown density (Figure 1A, 1B) during the summer months. Flowering of hydrilla was observed in late September through October, just prior to tuber and turion formation (Figure 1C).

During the fall months, starting in October 1994, an increase in stolon, tuber and turion densities occurred as these storage organs were produced for winter (Figure 1B, 1E). Increases in tuber density were also evident in the core data (Figure 1D). Other experiments (unpubl. data) have indicated that undisturbed hydrilla tubers will germinate in the latter part of July through August at this location. Evidence for this timing of tuber germination can be seen in the decrease in tuber numbers before new tubers are produced in the fall (Figure 1D, 1E). At the LAERF, hydrilla regrows in the spring from stolon and root crowns, rather than tuber germination. The optimum temperature for tuber germination has been shown to occur between 15 and 35 C (Haller et al. 1976), but temperature is not the only controlling factor. Following a drawdown in Rodman Reservoir, Florida, 80% of the hydrilla tubers were found to germinate (Haller et al. 1976). Tubers, as well as turions, are backup survival strategies for the primary plant. Thus all reproductive activities (sexual and asexual) occurred in the fall preceding plant senescence for winter.

An apparent disparity between tuber densities in core data (Figure 1D) and quadrat data (Figure 1E) occurs because plants produce tubers at the end of stolons, then stolons senesce and the tuber is left in the sediment. Tubers remain in the sediment, accumulating over time. The only tubers detected through quadrat sampling were those still firmly

attached to stolons, which were removed with the biomass samples. Tuber densities (up to 200 m<sup>-2</sup>) observed in our experimental ponds as sampled by coring are comparable to densities cited in the literature, though possibly at the low end of the range (Netherland 1997).

Hydrilla biomass was allocated principally to the above-ground shoots with a maximum dry weight of approximately 1200 g m<sup>-2</sup> occurring in June through August 1994 (Figure 2A, 2B). Since hydrilla is a canopy producer, during the warmer months, hydrilla effectively obtained sufficient light to extend carbohydrate production. As the water temperature increased, the biomass in the upper and lower stems increased (Figure 2A, 2B). Stolon, tuber and turion biomass decreased throughout the summer months before increasing in October as new storage organs were produced (Figure 2C).

The minimum for total nonstructural carbohydrate (% TNC) for all plant organs occurred in July 1994 and June 1995 for this study (Figure 2), when most reserve carbohydrates had been utilized by the plant for spring regrowth. From April through June, a steady increase in aboveground biomass was detected (Figure 2B) as the hydrilla rapidly utilized stored carbohydrates to reach the surface. Additional data at the LAERF (unpubl. data) have shown a low point for carbohydrate storage occurred in June, indicating that hydrilla populations can vary the point of low carbohydrate storage throughout a mid-summer time frame. A similar variation in the primary low point for carbohydrate storage was observed for Eurasian watermilfoil (Madsen 1997b). The time when hydrilla is expected to be most susceptible to a management technique is at the point in the seasonal cycle when the stored carbohydrates are at the lowest (Figure 2D, 2E). Without sufficient stored carbohydrates, the plant will recover more slowly, and the management technique may provide more effective control. In addition, mid-summer management of hydrilla may eliminate the formation of tubers and turions in the fall.

These phenological studies indicate that hydrilla utilizes carbohydrates stored in the root crown to promote rapid new spring growth. Carbohydrate storage in hydrilla is at its lowest point during the seasonal cycle in late June to mid-July, with storage predominantly in root crowns, stolons, and lower shoots. Tubers and turions are also storage organs, but provide for dispersal (turion) and long-term survival if the plant dies. These low points in carbohydrate storage may enable the aquatic resource manager to utilize specific timing of control for improved management of this weedy species.

## ACKNOWLEDGMENTS

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<sup>4</sup>National Oceanic and Atmospheric Administration (NOAA), 1994-1995, Local Climatological Data Monthly Summary, P.O. Box 610086, Dallas-Ft. Worth, TX.

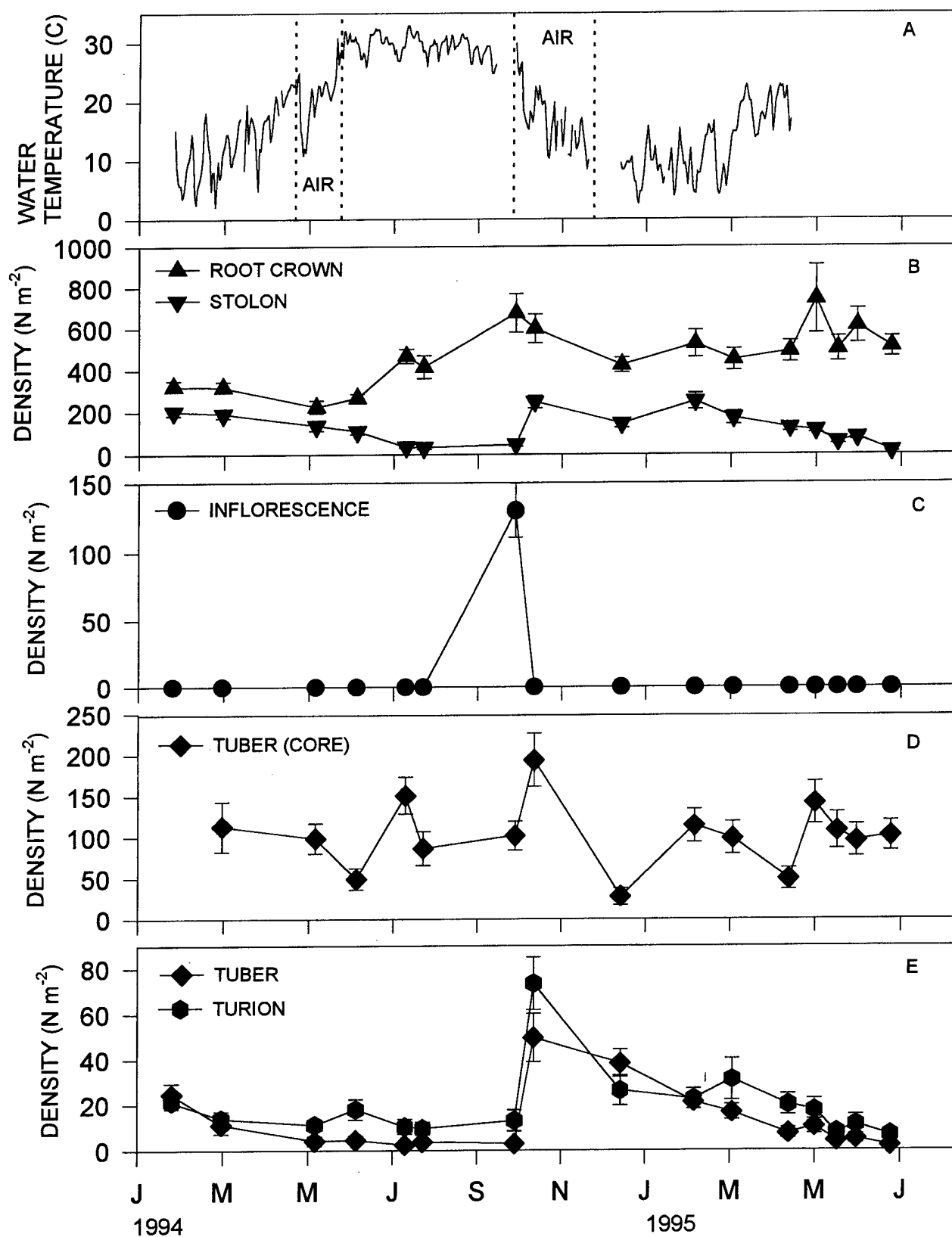


Figure 1. Hydrilla populations in experimental ponds in Lewisville, Texas, during 1994-1995. (A) Average daily water temperature in research ponds. The portions between the dotted lines indicate air temperature to replace missing water temperature data. (B) Density ( $N\ m^{-2}$ ) of hydrilla root crowns and stolons; (C) Density ( $N\ m^{-2}$ ) of hydrilla inflorescences; (D) Density ( $N\ m^{-2}$ ) of hydrilla tubers from sediment core data; and (E) Density ( $N\ m^{-2}$ ) of hydrilla tubers and turions from quadrat data. Bars indicate  $\pm$  standard error of the mean.

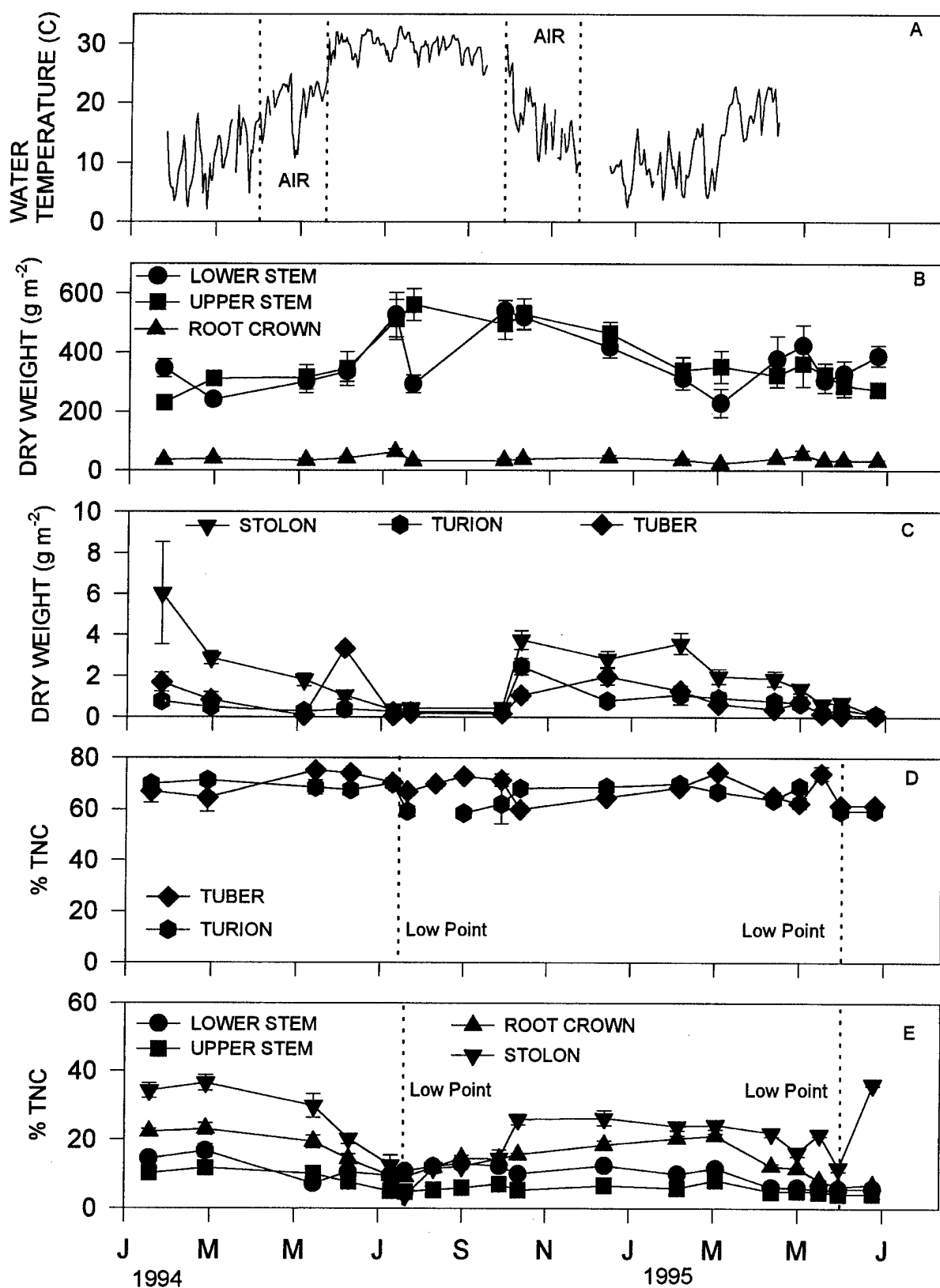


Figure 2. Hydrilla populations in experimental ponds in Lewisville, Texas, during 1994-1995. (A) Average daily water temperature for research ponds. The portions between dotted lines indicate air temperature to replace missing water temperature data. (B) Biomass ( $\text{g m}^{-2}$ ) of hydrilla lower stems, upper stems and root crowns; (C) Biomass ( $\text{g m}^{-2}$ ) of hydrilla stolons, turions and tubers; (D) TNC concentrations (% dry weight) of hydrilla tubers and turions. Dashed line indicates TNC seasonal low point; (E) TNC concentrations (% dry weight) of hydrilla root crowns, stolons, lower stems and upper stems. Dashed line indicates TNC seasonal low point. Bars indicate  $\pm$  stand error of the mean.

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# Techniques for Establishing Native Aquatic Plants

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## ABSTRACT

Man-made aquatic systems such as reservoirs are particularly vulnerable to infestations of weedy species because early in their existence they typically lack aquatic vegetation of any kind. Establishment of native aquatic plants in such systems could be an important deterrent to the spread of exotic weeds. This article describes a new Aquatic Plant Control Research Program (APCRP) work unit to develop methods for large-scale establishment of desirable native aquatic plants in man-made systems. The article discusses the need for work in this area, identifies the approach and research objectives, and describes early progress. An example project (Lake Conroe) is briefly described.

**Key words:** aquatic habitat, herbivory, lake restoration, plant production, plant propagation, revegetation.

## INTRODUCTION

**Justification.** Good integrated pest management requires that affected niches are never left unoccupied. An empty niche invites colonization by undesirable species and is a primary cause of recurring aquatic plant management problems. Man-made aquatic systems such as reservoirs are highly

susceptible to infestations of weedy species because, early in their existence, they generally lack aquatic vegetation of any kind. Many of these systems have extensive littoral areas capable of supporting diverse native plant communities that would enhance the structure and function of the entire ecosystem. Unfortunately, because natural establishment of native aquatic plant species is a relatively slow process, in many reservoirs nuisance exotic species often arrive first, establish, and spread to excess.

In this research we are developing methods for large-scale establishment of desirable native aquatic plants. This article briefly describes the concept of vegetating reservoirs by establishing founder colonies of desirable species and discusses production of plant propagules and planting methods.

**Reservoir situations.** Three situations occur in large, multi-purpose reservoirs that might interest managers in establishing native aquatic plants.

1. An absence of vegetation (or greatly limited quantities),
2. low species diversity, or
3. the reservoir is infested with nuisance exotic plants.

In the first two situations, we merely need to add native aquatic plants, while in the latter we must first address control of the nuisance exotic species.

Removal of established exotic weeds is covered adequately in other papers and will not be discussed here. In this paper we concern ourselves only with unvegetated reservoirs, including those from which aquatic weeds have been removed.

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Among reservoirs that can support aquatic vegetation, many are vegetated almost exclusively with exotic, weedy species. These weedy species are highly adapted for exploiting disturbed conditions (Smart and Doyle 1995). Several of the world's most problematic aquatic weeds are well-established in the United States, and these often arrive and establish before propagules of native species ever reach a new reservoir. Once established, in the absence of competition, exotic weeds often form large, monospecific beds and can prevent subsequent establishment of native plants, regardless of propagule availability.

One of the major vectors for the spread of exotic weedy species is human activity. The first sites colonized by exotics are often located near boat ramps, and transport by boats or boat trailers is considered one of the primary modes of spread of exotics from lake to lake. As an example, Texas Utilities Electric Company operates 16 power plant cooling lakes. Of these 16 lakes, 11 are open to the public and are infested with hydrilla (*Hydrilla verticillata* (L.f.) Royle.) while five of the lakes are closed and do not have hydrilla<sup>3</sup>.

In addition to accidental spread of exotics, there is an alarming number of cases where individuals or clubs have intentionally planted hydrilla in unvegetated reservoirs to "improve habitat". These individuals believe that exotic plants, such as hydrilla, benefit largemouth bass (*Micropterus salmoides*) and/or waterfowl.

**Benefits of aquatic plants.** Native aquatic plants provide valuable fish and wildlife habitat (Savino and Stein 1982, Heitmeyer and Vohs 1984, Dibble et al. 1996), improve water clarity and quality, reduce rates of shoreline erosion and sediment resuspension, and help prevent spread of nuisance exotic plants (Smart 1995). Water quality improvements arise from stabilization of deposited sediments (James and Barko 1995), filtration of suspended materials from the water, absorption of excess nutrients from the water (James and Barko 1990), and absorption (and sometimes detoxification) of some pollutants. Establishment of native aquatic plants can help prevent the spread of nuisance exotic plants directly by the principle of competitive exclusion (Smart 1995), and indirectly by eliminating the impetus for their intentional introduction by sportsmen.

**Rationale.** The aquatic plant communities that we observe in natural lakes have developed over hundreds of years. In many man-made reservoirs, there has not been enough time for a diverse community of native aquatic plants to develop. Because reservoirs are often constructed in areas that lack natural lakes, they may be remote from populations of aquatic plants that could serve as sources of propagules. As a result, many reservoirs receive only limited inputs of seed and other plant propagules.

Some reservoirs exhibit environmental conditions that may impede development of aquatic plant communities. Large water level fluctuations are common in many multipurpose reservoirs, and establishment of aquatic plants from seed or fragments will be difficult in such reservoirs. Small seedlings and developing young plants are especially vulnerable to conditions that place them in water that may be either

too deep to allow for adequate light penetration or so shallow as to expose them to either turbulence or desiccation.

Unvegetated reservoirs are often characterized by turbid waters and shifting, unconsolidated sediments. Small aquatic plants may not receive enough light to sustain photosynthesis rates needed for successful establishment under these conditions. Plants may also be adversely impacted by sediments coating the leaves or, in the worst cases, completely burying young plants.

Biotic disturbance represents a major factor that may affect establishment of aquatic plant communities. Fish and other organisms that feed or 'root' in sediments easily dislodge seedlings and other small, young plants. Also, herbivory by turtles, crayfish, insect larvae, muskrats, nutria, and beaver has been shown to be a significant factor affecting establishment and/or growth of submersed aquatic plant communities (Lodge 1991, Dick et al. 1995, Doyle and Smart 1995, Doyle et al. 1997). These animals are all highly mobile and many are widely distributed throughout river systems. Also, many of them are omnivores, so their presence is not entirely dependent on the prior availability of plants. As a result of their widespread distribution and mobility, these omnivores are generally present in sufficient numbers to prevent, or at least delay, establishment of aquatic vegetation. In some systems, grass carp (*Ctenopharyngodon idella* Val.) have been used to control aquatic weed infestations, and their continuing presence may prevent establishment of any aquatic plant species for many years (Van Dyke et al. 1984).

In summary, the problem—a lack of aquatic vegetation (particularly submersed aquatic vegetation)—can be attributed to three major factors:

1. A paucity of plant propagules,
2. adverse abiotic conditions, and/or
3. biotic disturbances.

## RESEARCH APPROACH

To overcome the above limitations, establishment of submersed aquatic plant communities in unvegetated reservoirs will require introduction of suitable plant propagules, into protected environments, at times and locations that will minimize adverse environmental conditions during early establishment.

Because many of our multipurpose reservoirs are quite large and have extensive littoral zones, it would be prohibitively expensive to plant even a small fraction of the ultimate aquatic plant habitat available. A more effective and practical approach is to ensure establishment of "founder colonies" in strategic locations within the reservoir and to rely on these colonies to produce the propagules that will ultimately vegetate the littoral zone of the entire reservoir (Smart et al. 1996). The successful spread of exotic species from single sites of introduction attests to the validity of the founder colony approach.

It is always tempting to use seeds to establish vegetation over large areal expanses. If the lack of vegetation was simply the result of a lack of plant propagules, seed could be a relatively easy and inexpensive method of introducing desirable species into the reservoir. However, as previously mentioned, turbid, unvegetated reservoirs are inhospitable environments for seedling establishment, and development of plant

<sup>3</sup>Gary Spicer, Texas Utilities Electric Company, Personal communication.

communities from seed may require a considerable length of time even in the presence of a steady input of seeds. The low probability of seedling establishment is reflected in the rarity of sexual reproduction as compared to vegetative reproduction in most submersed aquatic plant species (Les 1988, Titus and Hoover 1991, but see Brock 1983). In this regard it is interesting to note that the most problematic of the exotic submersed plant species (hydrilla, Eurasian watermilfoil (*Myriophyllum spicatum* L.), and *Egeria densa* Planch. in the U.S. and *Elodea canadensis* Michx. in Europe and Japan) very rarely or never reproduce by seed (Sculthorpe 1967, Aiken et al. 1979, Pieterse 1981, Reimer 1984, Haramoto and Ikusima 1988). Although considerably more effort is involved, the use of mature transplants or robust propagules (tubers, root crowns, etc.) may considerably reduce the time required to successfully establish founder colonies, particularly in inhospitable reservoir environments.

The founder colony approach (Smart et al. 1996) involves the establishment of small colonies of several aquatic plant species by planting transplants or robust propagules. These propagules are more tolerant of both abiotic and biotic stresses than seedlings or sprigs (Titus and Hoover 1991, Doyle and Smart 1993). Species are selected based upon past, current, and expected environmental conditions. Locations determined to be most suitable for a particular plant's

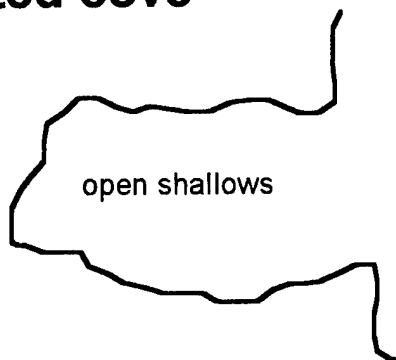
growth are chosen, and each species is planted within protected plots to reduce herbivory and biotic disturbance. Once successfully established, founder colonies will spread beyond their protective borders to adjacent, unvegetated areas of the reservoir (Figure 1). Ultimately, these founder colonies will provide a continuing source of propagules to the reservoir, eventually filling empty aquatic plant niches (Smart et al. 1996).

### PROPAGULE ACQUISITION

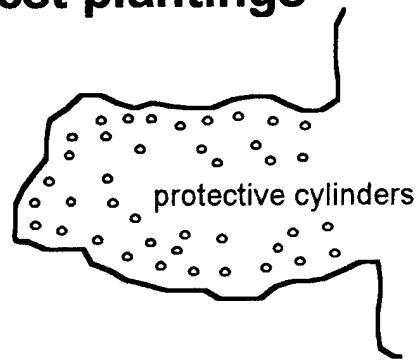
Propagules of some aquatic plant species may be purchased from commercial suppliers. However, many submersed species are not commercially available. To secure robust propagules of suitable aquatic plant species, producing planting stock by using locally-collected (and locally-adapted) plant materials may be preferable.

Large-scale restoration efforts require dedicated outdoor tanks or ponds for mass culture of plants. Plants may be grown to produce seed, tubers, stem fragments, or to be used as transplants. Tuber-forming species may be grown to produce tubers in containers held in large outdoor tanks or ponds. After the plants senesce, the containers can be removed from water and stored for several months until tubers are needed. Mature transplants can be produced by

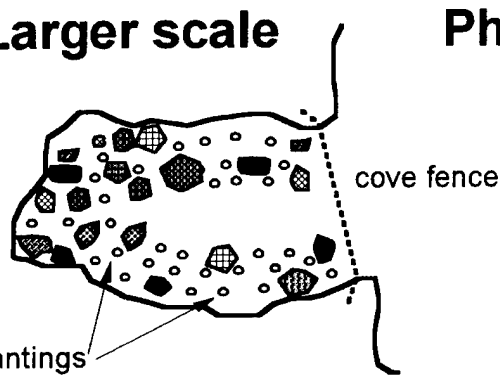
## Unvegetated cove



## Phase 1. Test plantings



## Phase 2. Larger scale



## Phase 3. Expansion

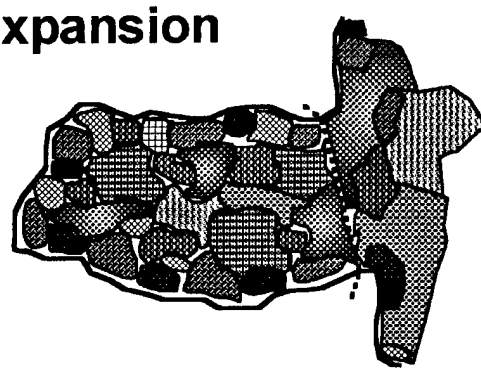


Figure 1. Diagrammatic representation of founder colony approach. Phase 1 involves planting of test plants within small protective exclosures. During the second growing season (Phase 2), a larger scale fenced area is constructed, if necessary, and additional plantings of the most suitable species are made. During the third and subsequent growing seasons (Phase 3), the founder colonies vegetate the rest of the reservoir.

growing plants in nursery pots held in large outdoor tanks or ponds. Smart et al. (1996) proposed that plant production requires the provisions of fertile sediments, low phosphorous water (<10 µg/L) to prevent excessive algal growth, moderate temperatures (20-28 C) and adequate light levels (35-65% of full sunlight).

## HERBIVORE PROTECTION

Establishment of new colonies of aquatic plants in unvegetated reservoirs requires protection from herbivores. This conclusion is based upon our experiences (Smart et al. 1996, Doyle et al. 1997) and those of others who have attempted to establish submersed aquatic plants in lakes and reservoirs in several states. We have used several types of protective enclosures, depending on the expected level of herbivory. Site visits, discussions with lake and fisheries managers, and trapping can provide preliminary estimates of the densities of herbivorous species that may be encountered.

1. **Individual plant protection**—A cylinder, 60 to 90 cm in diameter by 91 or 122 cm (3 or 4 ft) high, constructed from 2" by 4" mesh welded-wire fencing and anchored with 152- or 183-cm (5- or 6-ft) lengths of rebar. The cylinder can be closed at the top by cinching opposite sides together and securing with wire ties. This enclosure is designed to protect single transplants from larger omnivores such as adult turtles, carp, nutria etc. If protection from juvenile turtles and/or crayfish is needed, enclosures can be made from smaller mesh size material.
2. **Multiple plant protection**—A square cage, 150 or 180 cm (5 or 6 ft) on a side, constructed of 122- or 183-cm (4 or 6-ft) high, 1.5" mesh orange plastic construction fencing, rebar, and PVC piping (Smart et al. 1996). These enclosures are usually planted with four or five transplants and may be suitable for harsh environments where survival of an individual transplant may be in doubt. The larger area of the resultant population may also sustain a higher grazing pressure than would an individual plant unit. The smaller mesh size of the construction fencing also provides more complete protection from most herbivores and omnivores. An additional advantage is the high visibility of the material, making the plantings easy to find for monitoring and evaluation and also easy for boats to avoid. Drawbacks include greater expense and difficulty of construction and less durability in comparison with the welded wire mesh enclosure design above.
3. **Fenced plots**—Square or rectangular fenced areas measuring 3.5 m or greater on a side and constructed from 122- or 183-cm (4 or 6-ft) high, 2" by 4" mesh welded-wire fencing.
4. **Shoreline fences**—A three-sided modification of the above fenced plot design. These are irregular in size, extending from the shoreline out to, for example, the 1-m contour and then along that contour parallel to the shore. These are also constructed of 122- or 183-cm (4 or 6-ft) high, 2" by 4" mesh welded-wire fencing.
5. **Fenced coves**—Cove areas isolated from the main body of the reservoir by fences constructed of 2" by 4" mesh welded-wire fencing placed across the mouths of small coves.

The above small-scale enclosures (1, 2, and 3) can provide near-complete protection from herbivory if constructed of appropriate mesh size material and deployed properly. However, because enclosures 1 and 2 protect only a single, relatively small clump of plants, they may be most useful in situations where herbivory is low to moderate. Larger herbivore enclosures (3, 4, and 5) offer protection from omnivores such as carp and other rough fish. These are used in situations where rough fish population densities are expected to be high, or in reservoirs stocked with grass carp.

Because fenced coves and shoreline fences do not exclude herbivores that can move over land (turtles, nutria, muskrat, beavers), these may require a double-layer of herbivore protection (individual plant enclosure plus fenced cove or shoreline).

## IMPLEMENTATION

A diagrammatic representation of the founder colony approach is given in Figure 1. A suitable cove (one with an expanse of shallow water, suitable sediments, and a relatively protected location) is identified. Phase 1 involves planting and monitoring (over a full growing season) of test plants of a variety of species within small protective enclosures. Assuming suitable sediments, water quality, and water levels, these plants will establish and expand beyond their protective cages, depending on the level of herbivory. During Phase 1, the level of herbivory should be noted and, if possible, the sizes and types of herbivores.

In most unvegetated reservoirs, expansion of the plantings will require provision of a larger-scale protected environment such as a fenced cove. In Phase 2, those species performing best during Phase 1 should receive additional plantings. Phase 2 (if required) includes construction of a fence across the cove mouth to exclude carp and other rough fish in combination with additional plantings of selected or preferred species. Phase 2 should result in the successful establishment of founder colonies of several species. During Phase 3, the colonies expand to fill the niche within the fenced cove, and begin to spread into unprotected areas by vegetative and/or sexual modes of reproduction.

## LAKE CONROE EXAMPLE

*Background.* Shortly after its impoundment, Lake Conroe was invaded by hydrilla. This aggressive exotic plant soon choked the lake with dense mats of vegetation and the state of Texas approved a one-time stocking of 270,000 herbivorous exotic fish (grass carp) to control the growth of hydrilla in Lake Conroe. The grass carp quickly consumed all of the hydrilla and for over 15 years have prevented the establishment of aquatic vegetation of any kind. A multi-agency project involving state, local, and Federal organizations has been initiated to study and demonstrate methods for establishing native aquatic vegetation in the lake. Native plants would provide much-needed fish habitat and would help prevent a re-infestation of the lake by hydrilla.

*Project description.* The Lake Conroe Revegetation project consists of four phases: test plantings, larger-scale demonstration sites, development of a on-site plant production nursery, and full-scale implementation. The first two phases corre-

spond to Phases 1 and 2 described previously (Figure 1). In August of 1995 (Phase 1) test plantings were conducted at 15 locations in the lake. Plants were planted inside protective cages to determine which native plant species were best suited for conditions occurring in Lake Conroe. The test plantings also served as a gauge for evaluating the effects of the grass carp population.

**Results.** The three submersed species, American pondweed (*Potamogeton nodosus* Poir.), water star grass (*Heteranthera dubia* (Jacq.) Macm.), and wild celery (*Vallisneria spiralis* L.) readily established in the protective enclosures. Although each of these species exhibited repeated attempts to spread beyond the confines of the enclosures via vegetative growth, the grass carp effectively prevented any significant expansion.

Because grass carp were found to be a significant factor in preventing expansion from small-scale plantings, larger protected areas were employed in Phase 2. Six cove sites were selected from the 15 original sites and were fenced off in March of 1996. These sites received additional plantings of American pondweed (one site), water star grass (one site) or wild celery (four sites) in April, 1996. Single mature transplants were planted within individual plant protection cylinders at each of the sites. Site 1 received 30 American pondweed plants; Site 2 received 40 water star grass plants; and Site 5 received 20 wild celery plants.

We assessed survival and growth (expansion) bimonthly, in June, August, and September, 1996. Survival of the transplants was 97, 95, and 100%, for American pondweed, water star grass and wild celery, respectively. Expansion of the plants is shown in Figure 2. Both American pondweed and wild celery spread very rapidly, achieving mean colony diameters greater than 2.5 m. This indicates that planting on 3-m centers could provide nearly complete coverage in just a single growing season. Water star grass did not expand as rapidly as the other two species. The slower lateral expansion rate of water star grass was expected because this species

grows by proliferation of shoots within the root crown and spreads by fragmentation. We observed many new colonies of water star grass within the fenced coves. These new colonies likely resulted from shoot fragments that broke off, drifted a short distance, and rooted. These results indicate that establishment of founder colonies can be quite rapid. In addition to the three species directly planted, we also observed an abundant growth of annual species. Both musk grass (*Chara* sp.) and southern naiad (*Najas guadalupensis* Spreng.) were present as either plants and/or seeds in the transplant materials. These pioneer species benefitted from the protected environment and spread very rapidly.

## FUTURE RESEARCH

Research on methods of producing transplant materials (both at remote sites and within-lake) continues. Research on methods of protecting transplants from herbivory also continues. Several lake restoration projects have been initiated using the techniques described here. These include the following reservoirs: Arcadia Lake (Oklahoma), El Dorado Lake (Kansas), and Lake Livingston (Texas).

## ACKNOWLEDGMENTS

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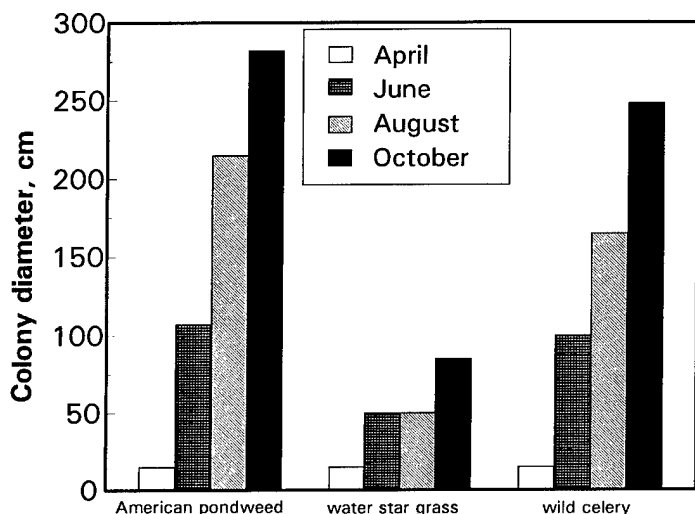


Figure 2. Vegetative expansion of individual transplants of American pondweed, water star grass, and wild celery in selected fenced coves in Lake Conroe during 1996. Values are means of 30, 40, and 20 replicate transplants, respectively.

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# Overview and Future Direction of Biological Control Technology

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## ABSTRACT

The Corps of Engineers (CE) biological control technology area had its beginnings in 1959 when the CE and the U. S. Department of Agriculture began a cooperative research effort. Since then, numerous insects and pathogens have been studied as potential agents for the management of target plant populations. Researchers have traveled to the countries of origin of six target plants (*Eichhornia crassipes* Mart. (Solms), *Alternanthera philoxeroides* (Mart.) Griseb., *Myriophyllum spicatum* L., *Pistia stratiotes* L., *Hydrilla verticillata* (L. F.) Royle, and *Melaleuca quinquenervia* (Cav.) S. T. Blake) to search for host specific agents. As a result, 13 insect biocontrol agents have been released as management tools for five of these targets. On average these projects have developed one agent every 2.9 years. The CE also has conducted pathogen biological control research using endemic pathogens. More recently the CE has begun classical biocontrol studies using exotic pathogens as potential agents of aquatic plants. Research in the near future will be directed at the management of submersed aquatic vegetation. The past successes will be used to assist in directing the program, however, new emphasis will be placed on the development of more effective evaluation procedures to document impact of the biological control agents.

*Key words:* Aquatic plants, insects, pathogens, exotic plants, classical biological control.

## INTRODUCTION

Exotic aquatic plants have caused significant problems in the United States since the late 1800's (Sanders et al. 1985). Water hyacinth (*Eichhornia crassipes* Mart. (Solms)), an aggressive floating plant native to South America, was introduced into the United States in 1884 and fifteen years later, was identified by the U.S. Congress as hampering the operation of navigable waterways in Florida and Louisiana (Cofrancesco 1996). Over time other aquatic plants, such as alligator weed, water lettuce, Eurasian watermilfoil, hydrilla, and melaleuca developed into problems in waterways of the United States.

Beginning in the early 1900's, three management technologies have been employed to regulate populations of noxious aquatic plants. Mechanical control methods were the first technology employed and included everything from the manual removal of plants to the development of specialized machines (Gopal 1987). The next management technology developed was chemical control which first used inorganic compounds, then progressed in the 1940's to organic compounds, such as 2, 4-D (Bose 1945, Gopal 1987) and, now employs improved products for plant management. The most recent technology developed was biological control which started in 1959 with cooperative research projects between the U.S. Army Corps of Engineers (CE) and the United States Department of Agriculture-Agriculture

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Research Service (USDA-ARS). Projects were designed to locate host specific phytophagous insects to manage noxious plant populations (Center et al. 1990).

Biological control technology uses two approaches to manage noxious plants. The first is the inundative or augmentative approach which utilizes endemic organisms at higher than normal population densities to inflict significant damage on target plant populations. Often this approach has been used with weakly pathogenic organisms where the releases of elevated quantities of the agents produce a decline in the target plant population. The other approach, classical biological control, uses host specific arthropods, nematodes, or plant pathogens from the target plants native range to suppress the populations of the introduced noxious plant (Harley and Forno 1992).

This paper will give an overview of the CE biological control technology for aquatic plants. It will examine the research conducted on both insect and pathogen biological control agents, identify the adjustment made in response to the 1996 funding reductions, and outline the future direction of this technology area.

## OVERVIEW AND CURRENT RESEARCH

Since early 1900's the CE developed management tools for chemical, mechanical, and biological control technology to regulate problem aquatic plants. Although chemicals and equipment for the chemical and mechanical technology areas typically were provided by outside companies, the CE conducted research to evaluate the effectiveness of these new chemicals or equipment. Using this research the companies eventually profited from the sales of their herbicides or removal equipment. This cooperation with industry allowed CE research dollars for chemical and mechanical technologies to be directed on evaluating tools and not be used in tool development. Biological control technology research differs from other technology areas in that the discovery, development, and distribution of potential agents are funded totally by participating agencies. Private companies found it difficult to profit from the development and release of biocontrol agents when they could not control agent spread and distribution. This situation required that the CE in conjunction with the USDA-ARS provide all the funding and personnel for discovery, development, and distribution of the biological control agents identified. These extra steps often required increased research cost and time for development of new agents.

### Insects

The use of insects as biological control agents for plants in the U.S. dates back to the 1902 release of *Aerenicopsis champi-oni* Bates on Lantana in Hawaii. This was followed by additional releases of insect biological control agents for klamath weed, musk thistle, and other exotic plants (Julien 1992). In 1959, alligator weed (*Alternanthera philoxeroides* (Mart.) Griseb.), a native of South America, was the first aquatic plant targeted for classical biological control research. A USDA laboratory was established in Argentina as part of the cooperative effort of the USDA and CE to identify host specific phytophagous insects to manage alligator weed (Coul-

son 1977). During the initial surveys more than 40 insects were found feeding on alligatorweed (Coulson 1977). As testing progressed, the number of potential agents was reduced to five insects and in the end three agents were approved for release in the United States beginning in 1964 alligatorweed flea beetle (*Agasicles hygrophila* Selman & Vogt), alligatorweed thrips (*Amynothrips andersoni* O'Neill), and the alligatorweed stem borer (*Vogtia malloi* Pastrana); (Cofrancesco 1988, Vogt et al. 1992).

Over the last 38 years, the CE and USDA have joined forces to conduct research on six exotic plants (alligatorweed, water hyacinth, water lettuce, hydrilla, Eurasian water-milfoil, and melaleuca) and have released 13 biological control agents. These achievements have required research to be conducted on six continents; development of three overseas research facilities in Argentina, Australia, and China; and utilization of numerous facilities in other regions of the world. Table 1 gives an outline of agent introduction over the years. On average, an agent has been released every 2.9 years. Examination of release dates indicates there is always a significant lag time between identification of a new target plant and introduction of the first biocontrol agent. Projects also can have lag times develop when research operations are reduced or moved. Overseas research is the cornerstone of a successful classical biological control program. If researchers stop putting agents into the evaluation pipeline, then we stop having new agents available to manage the target population. The research leader must balance agent input into the pipeline with other facets of a biological control program, such as host specificity testing, agent release, and field establishment, to ensure a balanced overall program.

With the 1996 reduction in funding for the Aquatic Plant Control Research Program (APCRP) adjustment in insect biological control work units occurred. Four work units dealing with insect biocontrol agents were consolidated into one work unit. Although research efforts and funding priorities were structured so that critical facets of the research operation were maintained, a 50% reduction in funding occurred in the areas of release, establishment, and distribution of agents and an 80% funding reduction occurred in overseas research.

The primary focus of the consolidated insect biological control work unit was establishment and distribution of hydrilla biocontrol agents. Apparently, some agents approved for release on hydrilla will have little if any management value in the U.S. The tuber feeding weevil appears to be effective only in areas where hydrilla is dewatered, and tubers are exposed in the mud flats. *Hydrellia balciunas* Bock, an ephydrid fly from Australia, has been released at a number of locations in three states, however, the establishment of field populations has been difficult (Grodowitz et al. 1993). Efforts to widely establish the stem bore weevil (*Bagous hydrillae* O'Brien) have continued, however, only limited field populations of the agent have been documented (Grodowitz et al. 1994). Under laboratory and greenhouse conditions, the population of *Hydrellia balciunas* and *Bagous hydrillae* develop rapidly. The reason for difficulties in establishing field populations of these agents should be investigated.

*Hydrellia pakistanae* Deonier has been the most successful of the biocontrol agents released for hydrilla. It is widely dis-

TABLE 1. INFORMATION IS PRESENTED FOR EACH TARGET PLANT. THE TABLE IDENTIFIES THE YEAR THAT THE PROJECT STARTED ALONG WITH THE YEAR THE INSECT SPECIES WERE FIRST RELEASED IN THE UNITED STATES. THE NUMBERS INDICATE THE NUMBER OF INSECT SPECIES RELEASED DURING A SPECIFIC TIME PERIOD.

Years	ALLIGATORWEED (started 1960)	WATERHYACINTH (started 1960)	WATERLETTUCE (started 1982)	HYDRILLA (started 1980)	MELALEUCA (started 1987)	TOTAL Species Released
1960-61						
1962-63						
1964-65	1					1
1966-67	1					1
1968-69						
1970-71	1					1
1972-73		1				1
1974-75		1				1
1976-77		1				1
1978-79						
1980-81						
1982-83						
1984-85						
1986-87			1	2		3
1988-89				1		1
1990-91			1	1		1
1992-93						
1994-95						
1996-97					1	1
Total	3	3	2	4	1	13

tributed in Florida and has been released in four other states. In pond studies conducted at the Tennessee Valley Authority (TVA) facility, this agent proved it could overwinter in this northern range (Grodowitz et al. 1995). In addition, the TVA study showed that when large fly populations developed, the plants declined (Grodowitz et al. 1995). Once a sufficient density of individuals develops at field sites, studies will be undertaken to evaluate agent impact and compare with the TVA pond study.

Overseas and quarantine research efforts have been reduced significantly due to the funding reductions instituted in 1996. However, even under these reduced levels researchers have identified a number of agents associated with hydrilla in Thailand (Ted Center, USDA-ARS, personal communication). These initial survey efforts found two new weevil species and an undescribed ephyrid fly feeding extensively on hydrilla. Research efforts in China and India prior to funding reductions identified new potential agents for both Eurasian watermilfoil and hydrilla (Bennett 1993, Bennett 1994). Some of these agents are being maintained in quarantine until funding allows the completion of their host specificity testing. Along with these overseas efforts, Habeck (1996) completed work on the three Australian moths that feed on hydrilla. All of the moths produce damage of various degrees to hydrilla but none appear to possess the host specificity level necessary for release in the U.S.

## Pathogens

Pathogen agent development for biological control of noxious plants has followed a decidedly different pattern from that of insect agents. When the CE entered into pathogen biological control research in the 1960's, the only available approach was using endemic pathogens in an inundative strategy. Initiation of classical pathogen biological control research was not an option because a plant pathogen

quarantine facility did not exist in the U.S. until 1971 (Melching et al. 1983). In addition, it was extremely difficult to obtain approval for exotic pathogen releases. Between 1971 and the present, only *Puccinia chondrillina* Bubak and Syl., introduced in 1976 for management of skeleton weed (*Chondrilla juncea* L.) in the western U.S. (Supkoff et al. 1988, Julien 1992), have been approved for release in the U.S. Because of these factors, the CE has focused its pathogen biocontrol research on endemic pathogens for management of noxious aquatic plants.

The inundative approach was used with endemic fungal species *Cercospora rodmanii* Conway on *Eichhornia crassipes* (Freeman et al. 1981, Theriot et al. 1981) in the 1970's and a Massachusetts strain of *Mycocleptodiscus terrestris* (Gerg.) Ostaz-eski on *Myriophyllum spicatum* L. in the late 1980's (Gunner 1983, Gunner et al. 1991). This strategy applied the pathogen at rates that would overwhelm plant defense mechanisms resulting in a disease epidemic and reduction in biomass similar to that achieved with the use of herbicides. Formulation technology during this period was a new field; incorporation and preservation of living biological organisms into inert biocarriers was still in its infancy. In addition, formulation development was directed toward the needs of terrestrial plant pest problems ignoring the special needs of formulations for use in an aquatic environment. Neither formulations of the fungal pathogens proved effective in field tests because the formulations were inadequate. Further development was then halted.

Research continued using endemic pathogens for biological control of hydrilla even after the 1996 funding reductions. The Texas strain of *Mycocleptodiscus terrestris* has shown great promise as a biological control agent for hydrilla in laboratory, greenhouse, and field trials. To be marketable as a biological control agent, the fungus must be formulated, perform well in an aquatic system, have a shelf life, and be



applied easily using conventional equipment. Progress has been slow but recent developments in encapsulation technology have demonstrated that viability of living organisms can be retained in a dry formulated product. Recent work with the newly formed company Trans America Product Technology (TAPT) has been extremely encouraging. Incorporating the fungus into a patent pending biocarrier, Biocar 405, has resulted in a formulation which in initial testing has proved efficacious, operational in an aquatic environment, and viable on the shelf for up to three months.

Pathogen biological control efforts continue to research the use of endemic pathogens but an important dimension has been added through the initiation of foreign exploration to find agents that can be used in a more classical approach. Overseas surveys for pathogens of aquatic plants began in 1994 in Europe through a contract with the International Institute of Biological Control (IIBC) and in China as a cooperative research effort with the USDA. Between 1994 and 1996, IIBC researchers surveyed 200 sites in 12 countries in western Europe for pathogens of Eurasian watermilfoil, and collected 290 isolates (Harvey and Evans 1997). During 1994 and 1995, more than 200 isolates were obtained from hydrilla and Eurasian watermilfoil tissue in China and preserved in long term storage at the National Cancer Institute, Ft. Detrick, Frederick, MD (Shearer 1997). Screening of the isolates for potential pathogens is being conducted at the USDA-ARS Foreign Disease Weed Science Research Facility.

Recent events in the permitting process in the United States have made the release of exotic pathogens more realistic. Within the last few years, the USDA has been reevaluating the criteria for release of exotic pathogens. Under the new federal and state guidelines pathogen releases should become more frequent.

### FUTURE DIRECTION

Biological control research efforts must build on past success and move forward to predict the long term impacts of agents that have been released. We need to maintain a technology area that is responsive to the needs of our customers and visionary enough to identify potential problems before they are excessive. We must educate our customers on how to effectively use the biocontrol resources they have available either alone or in conjunction with an integrated management program. By educating and interacting with our customers, we can become aware of problems as they develop and be in a better situation to adjust our research efforts to meet their needs. Our future research emphasis will be placed on developing agents for submersed aquatic macrophytes and post evaluation of agents already released.

Pathogen biological control research will be conducted on both endemic and exotic pathogens. Research on endemic pathogens will focus on the formulation of *Mycotodiscus terrestris* (Mt) for use on hydrilla. Classical biocontrol research of pathogens will continue to survey and develop host specific exotic organisms for submersed vegetation. Overseas research efforts to find pathogens of hydrilla and Eurasian watermilfoil have covered only a small portion of the range of these plant species (to date, western Europe and a few areas of north and central China). Areas within the native range of hydrilla and Eurasian watermilfoil that have

not been surveyed for pathogens include eastern Europe, most of Asia, Australia, and Africa. In the past year, contacts have been made with cooperators in eastern Europe, Russia, and southeast Asia who have indicated they would assist with survey efforts in those regions. Russian scientists in St. Petersburg have collected some pathogens of Eurasian watermilfoil and are willing to cooperate in their evaluation as well as in additional exploration. Availability of overseas cooperators combined with more realistic guidelines for release of exotic pathogens make research in this area a timely, feasible, and worthwhile effort.

The release and establishment portion of the insect biological control research program will change its direction. Instead of attempting to obtain broadest possible distribution of the agents, we will attempt to make mass releases of *Hydrellia pakistanae* on large lakes to develop the same kind of situation that was observed in the TVA study. We also will begin to institute laboratory studies directed at determining why particular agents have difficulty in establishing field populations and try to better understand how each agent is impacting their target plant.

Along with the above studies, research will be conducted to develop more effective evaluation procedures to document impact from biological control operations. These evaluation procedures will be used to document the historical impact of biocontrol agents and evaluate introduction of the new agents. A review of the impacts of insect biological control has been long overdue. This information can be presented to our sponsors, to assist in their education of how to employ the biocontrol resources that are available.

Overseas insect studies will address areas of the world that have not been explored and focus on developing agents for hydrilla and Eurasian watermilfoil. Many of these overseas efforts will be conducted in conjunction with pathogen research wherever possible. The wealth of the agents identified in Thailand from a relatively short trip indicates that a number of potential insect agents still exist. Special efforts will be made to expedite the introduction of these new agents, particularly the weevils which usually have a limited host range, a key requirement for a biocontrol agent.

Research also will be directed to determine the feasibility of using endemic insects as biological control agents of Eurasian watermilfoil. The CE funded much of the laboratory and field research that has been conducted on the native weevil *Euhrychiopsis lecontei* Dietz (Creed and Sheldon 1993). The results of these studies along with studies that have been conducted by researchers in Minnesota make this approach worth investigating for Eurasian watermilfoil management.

The Biological Control Research Technology Area for the CE will attempt to balance all research efforts identified to ensure a comprehensive program that meets the needs of our sponsors. Although priorities will be established once the funding for the program has been identified, management of submersed aquatic vegetation will be the primary focus of the technology area in the future.

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# Biological Control of Hydrilla Using an Endemic Fungal Pathogen

J. F. SHEARER<sup>1</sup>

## ABSTRACT

Prototype formulations of the hydrilla fungal pathogen, *Mycileptodiscus terrestris* (Gerd.) Ostazeski, were tested for efficacy against dioecious *Hydrilla verticillata* (L.f.) Royle in test tube, column, and tank studies. The formulation was produced by Trans America Product Technology, Inc. (St. Charles, MO) by incorporating the fungus into a patented biocarrier, Biocar 405. Initial test tube studies demonstrated that both granular and caplet formulations induced disease ratings of 3 and 4 on excised hydrilla shoot tissue at two weeks post inoculation. Low, medium, and high dosage rates of the granular formulation applied to rooted hydrilla in 12 L columns reduced shoot biomass at four weeks post application by 87.7, 94.8, and 99.2% respectively compared to untreated controls. In tank studies a granular formulation reduced shoot biomass of hydrilla grown in 1700 L tanks by 97.5% at four weeks post application.

**Key words:** *Mycileptodiscus terrestris*, *Hydrilla verticillata*, mycoherbicide, formulation.

## INTRODUCTION

The endemic fungal pathogen, *M. terrestris* is a potential biological control agent for hydrilla as demonstrated by laboratory, greenhouse, and field trials (Joye 1990, Joye and Cofrancesco 1991, Joye and Paul 1992, Shearer 1996, Shearer 1997). Four to seven days following inoculation with the pathogen, the first disease symptoms appear as a chlorosis of hydrilla leaf tissue. Within two weeks, leaves and stems become flaccid, lyse, and float to the water surface. Observations from a histological study of the infection process suggested that enzymatic action allows pathogen ingress into the cells of the lower epidermis (Joye and Paul 1992). Following entry, the fungus colonizes host cells resulting in a disruption of cell walls and subsequent cell death.

*M. terrestris* makes an ideal candidate for inundative biological control because the pathogen is endemic, the disease episode is of short duration, and the fungus does not persist in hydrilla debris or plant tissues in the field (Shearer 1996). The approach also known as the mycoherbicide strategy utilizes pathogens in much the same way as chemical herbicides (TeBeest 1993). A pathogen is formulated and applied to a target host population in volumes that achieve control within an allotted period of time. That the strategy can be successful has been documented by the development of formulated

*Colletotrichum gloeosporioides* f.sp. *aeschynomene* (COLLEGO) to control northern jointvetch in rice and soybeans in the southeast United States (TeBeest 1993) and formulated *Phytophthora palmivora* (DeVine) to control stranglervine in citrus groves in Florida (Charudattan 1991).

To date, formulation research on fungal biocontrol pathogens has primarily been directed toward development of mycoherbicides to control terrestrial weeds on agricultural land. One of the major considerations in formulation effectiveness has been the retention of a wetting or dew period to prevent desiccation of the fungus until infection can be established within host tissues (Hasan and Ayres 1990). While retention of fungal viability is a consideration in the development of a mycoherbicide for use on submersed macrophytes, dew period in an aquatic system is not of major concern. To be effective in an aquatic environment, the shape, size, and buoyancy of a formulated fungus may need to be adjusted to allow for dispersal and adequate coverage of the target plant in an aqueous medium.

Formulation development encompasses several steps including manipulation of the fungus, design of biocarriers, and incorporation of the fungus into the biocarrier. Biocar 405, a patented EPA approved biocarrier, developed by Trans America Product Technology, Inc. (TAPT, St. Charles, MO) was tested as a potential biocarrier for *M. terrestris*. Because the biocarrier had proved effective in dispersal of a mosquito pathogen in an aquatic habitat, it was thought that it might work equally well with the hydrilla pathogen. The performance of the prototype formulation of Biocar 405 and *M. terrestris* was evaluated in laboratory and greenhouse experiments on excised and rooted hydrilla.

## MATERIALS AND METHODS

Seed cultures were prepared by plating *M. terrestris* onto potato dextrose agar plates (PDA) (Difco Laboratories, Detroit, MI). The cultures were incubated in the dark at 28 C for seven days. Plugs 4 mm in diameter were cut from the leading edge of the fungal colony. Five plugs of fungal mycelium were added to 1 L Erlenmeyer flasks, each containing 500 ml of modified Richard's V-8 juice broth (glucose, 10 g; KNO<sub>3</sub>, 10 g; CaCO<sub>3</sub>, 3 g; V-8 juice (Campbells), 200 ml; H<sub>2</sub>O, 800 ml). The flasks were placed on a platform shaker (New Brunswick, Edison, NJ) and agitated at 200 rpms. After six days, the mycelial mat was filtered through four layers of cheesecloth, suspended in 100 ml of sterile water, and comminuted in a blender for 30 sec. Dilutions of the fungal suspensions were plated onto Martin's agar (H<sub>2</sub>O, 1 L; agar, 17 g; KH<sub>2</sub>PO<sub>4</sub>, 0.5 g; K<sub>2</sub>HPO<sub>4</sub>, 0.5 g; MgSO<sub>4</sub>·7 H<sub>2</sub>O, 0.5 g; peptone, 0.5 g; dextrose, 10 g; yeast extract, 0.5 g; rose bengal,

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0.05 g; streptomycin sulfate, 0.03 g) to determine propagule density. The above procedures consistently result in a slurry with a colony forming unit count (cfu) of  $1 \times 10^6$  colony forming units (cfu)/ml.

The fungal slurry was shipped overnight in an ice-cooled chest to TAPT Inc. for processing. The fungus was incorporated into the biocarrier, Biocar 405 (Biosorb, Inc., Belle Glade, FL). For preliminary evaluation of formulation performance on hydrilla, the matrix was extruded as dry granules and caplets. For testing in columns and tanks, a granular form of the formulation was used.

The number of cfu/g of formulated fungus was determined by suspending a 0.5 g subsample of dry granules in 10 ml of sterile water and plating a series of dilutions. After four days incubation at room temperature, *M. terrestris* colony counts were determined by visual examination of the plates.

### Test tube studies

Sprigs of hydrilla 15 cm in length were collected from greenhouse hydrilla stock cultures, thoroughly washed in tap water, and placed in test tubes containing 60 ml of sterile water. An approximate 1 g dose (800 cfu/ml) of granules or one caplet of the formulated fungus was added to the water and allowed to dissipate over the plant material. Treatments were replicated five times. The test tube cultures were placed in a 26 C incubator set to a 12/12 light/dark cycle. After two weeks the plants were visually examined and rated for disease damage (0 = no damage; 1 = slight leaf chlorosis; 2 = general chlorosis of leaves and stems; 3 = leaves and stems chlorotic and flaccid; 4 = total plant collapse). Subsamples of the granules and caplets were kept cool and plated weekly onto Martin's agar to assess fungal viability. The subsamples were visually assessed for presence of *M. terrestris* colonies.

### Column studies

Clear acrylic columns (76 cm tall by 13.7 cm wide) (12 L) were used for small-scale greenhouse testing. Thirty-two-ounce plastic cups filled three-fourths full with lake sediment amended with ammonium chloride (0.5 g/L) and Esmigran (1.75 g/L) were overlain with five cm of tap-water-washed silica sand. Three 15-cm apical sprigs of hydrilla were planted in the sediment and the cups placed in the bottom of the column. Twelve liters of nutrient solution (Smart and Barko 1985) were added to each column. The columns were aerated and maintained at  $25 \pm 1$  C in an environmental chamber (Conviron, Pembina, ND). Plants were allowed to grow approximately four weeks before testing was initiated. Five, 10, and 20 g of formulated fungus were applied to the surface of the water. Allowing for dilution in the 12 L columns, the effective rates were approximately 42, 83, and 167 cfu/ml respectively. Low and medium-dose treatments of the fungal slurry were applied at rates of 5 and 10 ml (400 and 800 cfu/ml) respectively. Each treatment was replicated three times. After four weeks, three stem pieces 2 cm in length were collected from plants or floating plant tissue in each column, surface sterilized in a 10% bleach solution (0.5% NaOCl) for 1 min, rinsed in sterile water, and plated onto Martin's agar to determine presence/absence of the fungus in the plant tissue. The remainder of the aboveground biomass from

each column was harvested and dried to a constant weight at 60 C.

### Tank studies

Tanks (160 cm in diam by 92 cm deep, approximately 1700 L) were used for testing formulated *M. terrestris* on a larger scale outdoor study. Lake sediment was amended as described above. Plastic containers (36 cm by 30 cm by 13 cm) were filled with sediment to a depth of 8 cm and overlain with 4 cm of tap-water-washed silica sand. Twenty-five apical sprigs of hydrilla 15 cm in length were planted in each container. Ten containers were placed in each of six tanks and the tanks filled with nutrient solution (Smart and Barko 1985). The plants were allowed to grow until they reached the water surface and formed a canopy. The granular formulation was dispersed in 400-g doses over the water surface of treated tanks and allowed to naturally dissipate onto hydrilla tissue. The effective application rate allowing for dilution was 23 cfu/ml. Treatments were replicated three times. After four weeks, aboveground biomass was harvested from treated and control tanks. Small subsamples of plant stem tissue 2 cm in length were taken from each sample to assay for presence/absence of the fungal component. The remaining biomass was dried to a constant weight at 60 C.

Data were analyzed using t tests and one-way analysis of variance (ANOVA). A confidence level of  $P = 0.05$  was used to determine statistical significance. Analyses were performed with the Sigma Stat program for the Windows™ operating system 1995 (SPSS Inc., Chicago, IL).

## RESULTS AND DISCUSSION

One of the most important features required in the design of a successful formulation of *M. terrestris* is plant surface area coverage because each point of contact between a formulation particle and hydrilla tissue is a potential site of ingress by the fungal pathogen. The test tube studies indicated that the best coverage over plant surfaces was achieved using the granular formulation. As the granules absorbed water, they disintegrated into small particles that became lodged on plant surfaces. In contrast, the caplets disintegrated into fine particles that either lodged on surfaces, agglutinated in masses, or collected in the bottom of the test tube. The uneven distribution and wastage of particles eliminated the caplet from further evaluation as a potential formulation of *M. terrestris*. Another area of concern in formulation development was retention of viability and virulence. Physical processes of extruding and drying the formulated product had minimal effects on fungal virulence and viability.

### Test tube studies

At two weeks post inoculation, damage on hydrilla using granules or caplets was assessed a disease rating between 3 and 4 (i.e. the hydrilla sprigs were severely chlorotic and flaccid or collapsed in the bottom of the tube). Viability was confirmed through consistent fungal retrieval from dried granules and caplets up to three months following production at which time the supply was depleted.

TABLE 1. MEAN DRY WEIGHT BIOMASS OF HYDRILLA SHOOT TISSUE COLLECTED 4 WEEKS POST APPLICATION WITH *M. TERRESTRIS* SLURRY OR *M. TERRESTRIS* FORMULATION.

Treatment	Control	Slurry 5 ml	Slurry 10 ml	Granules 5 g	Granules 10 g	Granules 20 g
Dryweight (g)	13.91a <sup>1</sup>	1.91b	0.76b	1.71b	0.73b	0.10b

<sup>1</sup>Values followed by a different letter are significantly different.

### Column studies

A fungal slurry of *M. terrestris* mycelia rated at  $1 \times 10^6$  cfu/ml applied at 5 ml and 10 ml to rooted hydrilla reduced shoot tissue 86.3 and 94.5% respectively by four weeks post inoculation (Table 1). Comparable reductions in hydrilla shoot biomass, 87.7 and 94.8%, were achieved with applications of 5 g and 10 g respectively of *M. terrestris* formulation rated at  $1 \times 10^5$  cfu/ml. At the highest rate of application (20 g), the formulated fungus reduced biomass by 99.2%.

*M. terrestris* was recovered from hydrilla shoot tissue four weeks post application with the fungal slurry and from the 5- and 10-g dose treatments of the formulation. Insufficient plant material remained in the 20-g formulation treatment for analysis.

### Tank studies

By week one of the tank study, disease symptoms were apparent on hydrilla. Leaves were chlorotic and there was evidence of stem fragmentation. By week three of the study, the epidemic had waned and little hydrilla tissue remained in the treated tanks. Because the tanks were outdoors, there was some concern that insects might transmit fungal inoculum to untreated hydrilla, but at the four-week-post inoculation harvest, *M. terrestris* was not recovered from hydrilla shoot tissue collected from treated or control tanks.

The procedures used in processing and drying of the formulation granules, although potentially harmful to fungal mycelia, did not severely affect *M. terrestris* viability. Formulation of the fungal slurry rated at  $1 \times 10^6$  cfu/ml resulted in an approximate log reduction in cfu counts. Four weeks post inoculation, hydrilla shoot biomass was reduced 97.5% compared to untreated controls (Table 2). The effective rate of fungal inoculum used in each tank (24 cfu/ml) was 66% lower than the effective rate of the low dose treatment of the column study (42 cfu/ml). Good coverage over plant tissues resulting from excellent dispersal characteristics of the granules meant less inoculum was required to achieve the desired biomass reductions. Each contact point of a formulation particle with hydrilla tissue was a potential site of fungal growth and subsequent invasion. Because the fungus does not ramify extensively within host tissue following invasion, multiple points of contact are necessary to promote a disease epidemic. In previous tank studies, inoculum applied in the form of a fungal slurry reduced hydrilla shoot biomass by 85% (Shearer 1994). A liquid inoculum has a tendency to

TABLE 2. MEAN DRY WEIGHT BIOMASS OF HYDRILLA SHOOT TISSUE FOLLOWING APPLICATION OF *M. TERRESTRIS* FORMULATION.

Treatment	Control	Granules
Dry weight (g)	391.2a <sup>1</sup>	9.66b

<sup>1</sup>The mean values of the inoculated tanks were significantly different from those of the control tanks as determined by the t test.

remain suspended in the water lessening the potential for physical contact between pathogen and host that is necessary for cell invasion.

Biocar 405 appears to be an excellent biocarrier for the fungus *M. terrestris*. The formulation process preserves pathogen viability and virulence. The dry granule provided good coverage over hydrilla plant surfaces and should be easily adaptable for application with conventional spray equipment. Additional testing on a larger scale under field conditions will confirm if the formulation has potential as a marketable mycoherbicide for hydrilla.

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# An Active Approach to the Use of Insect Biological Control for the Management of Non-Native Aquatic Plants

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## ABSTRACT

Today, the use of insect biological control for the management of aquatic and wetland plants is typically a rather passive procedure from the viewpoint of resource managers. Insects are released, usually by researchers, with little or no direct input or effort by management personnel. However, the effectiveness of biological control could be enhanced if resource managers took an active role in its use. Four steps should be utilized in order to achieve a more active approach to the use of biological control. These include gaining an understanding of the insect agents, initiating yearly surveys to determine insect population levels and immediate and long-term impact, supplementing the insect populations if surveys reveal low numbers, and developing integrated procedures to minimize impact of the varied management techniques to one another. Utilizing a more active approach increases the awareness of biological control techniques and should result in increased effectiveness.

**Key words:** Information systems, integrated pest management, waterhyacinth, alligatorweed, hydrilla, waterlettuce.

## INTRODUCTION

Over the last 38 years, the use of biological control for the management of non-native aquatic and wetland plant species has expanded tremendously. Since 1959, 18 insect species have been released for the management of several species of aquatic and wetland plants including alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb.), waterhyacinth (*Eichhornia crassipes* Mart. (Solms)), waterlettuce (*Pistia stratiotes* L.), hydrilla (*Hydrilla verticillata* (L.f.) Royle), purple loosestrife (*Lythrum salicaria* L.) and melaleuca (*Melaleuca quinquenervia* Cav. Blake). Currently over 20 insect agents are being tested in overseas laboratories and quarantine facilities to determine impact to these same plant species. In addition, active release and monitoring programs for insect agents of hydrilla, waterlettuce, melaleuca, and purple loosestrife continue (personal communication Dr. T. Center, USDA, ARS, Fort Lauderdale, FL, Dr. G. Buckingham, USDA, ARS, Gainesville, FL, and Dr. Al Cofrancesco, WES, Vicksburg, MS).

The first practical use of host-specific insect agents for the management of problem aquatic plants in the United States

began in 1964 with the release of the first of three insect species for the management of alligatorweed (Coulson 1977). In the following years, dramatic and often complete control of alligatorweed, comparable to that observed with herbicides, was recorded at various sites across the southeastern US (Coulson 1977, Vogt et al. 1992, Cofrancesco 1988). By releasing a small number of insects (usually less than 500) at a site a noticeable suppression or complete elimination was achieved in only a matter of months. Even more significant, declines in alligatorweed infestations continued for years with little additional input from resource managers in the form of supplementary insect releases or the use of more traditional management techniques. The only exceptions occurred at the more northern limits of the US alligatorweed distribution. In these areas continued releases were necessary because the insect populations did not persist from year to year, apparently due to continual sub-freezing conditions. The alligatorweed biocontrol program was a remarkable success and was, in part, responsible for continued interest and enthusiasm for research into the use of biological control for other aquatic and wetland plant species.

Waterhyacinth was the next plant species targeted for biological control beginning in 1972. Three agents, including two weevil species in the genus *Neochetina* and the moth *Sameodes albitalis* Warren, were released. Active participation by resource managers was the norm in the early years of waterhyacinth biocontrol and cooperative projects between state and federal agencies were initiated. One of the better publicized was the "Large-Scale Operations Management Test (LSOMT)" in Louisiana (Sanders et al. 1985). This project was a joint cooperative venture between the US Army Engineers Waterways Experiment Station (WES), the US Army Corps of Engineers New Orleans District, and various state agencies, to initiate releases over large areas with subsequent evaluation of the insect and pathogen biocontrol agents of waterhyacinth. It was soon realized, however, that the control achieved with the waterhyacinth insects was significantly different than that observed for alligatorweed. While large-scale reductions and virtual elimination of waterhyacinth were observed at some sites, the more typical scenario involved reduction of plant height, decrease in flowering (i.e., number of seeds produced), a decrease in the seasonal growth of the plants, and impacts that took years instead of months to occur (Center et al. 1990).

These impacts are a decided benefit. For example, reduction of plant height allows easier access by various forms of transportation including those associated with herbicide

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applications. Cost of chemical treatments is reduced since smaller plants require less herbicide for control. In addition, decreased seed production reduces future plant problems, especially in those areas where drought and subsequent water level fluctuations promote the growth and development of waterhyacinth seedlings. Insect feeding also decreases the production of daughter plants by destroying the apical meristems. This in turn diminishes yearly plant production, effectively reducing population size. Such is the case in Louisiana, where seasonal growth was reduced from a high of over 400,000 hectares per year to lows of only about 80,000 hectares (Center et al. 1990).

Unfortunately, the tremendous success with alligatorweed apparently fostered the unrealistic expectation by resource managers that the use of biological control technology always results in complete and long-lasting suppression or elimination of the target plant species. However, the type of "complete" control observed in the case of alligatorweed is not observed for the majority of insect biocontrol agents (Harley and Forno 1992, Hoffmann 1995). In most cases, when an insect biological control agent is used for weed management, measurable control occurs only after a period of years, not months, as was observed for alligatorweed. In addition, the complete elimination of the target plant is not anticipated, but rather the growth potential, seed production, or plant stature is impacted, thereby leading to long-term decreases in the plant infestation; exactly what was observed for waterhyacinth. Biocontrol is a suppression technology that, in most cases, reduces population growth and stresses the target plant. Reduced plant vigor coupled with adverse environmental conditions and/or the integrated use of other management options, results in smaller population size of the target plant, hopefully below what is considered the problem threshold.

Just as in the case of herbicides and mechanical control, the use of biological control requires, at the minimum, some continued input from resource managers to achieve measurable impact. The passive approach taken by resource managers today may be the result of what they observed with the application of the alligatorweed agents; i.e., they expected that all biological control procedures would take the same minimal input to achieve such outstanding success. It may, however, be related to the less than expected results observed with the waterhyacinth agents in comparison with alligatorweed. Whatever the reason, today almost all of the applied uses of biocontrol, including releases and subsequent monitoring, are performed by biological control researchers, a daunting task especially considering the vast acreage's associated with even one target plant species. Only limited expenditure of time and energy are put forth by resource managers.

Because of these misconceptions and the more passive role taken by resource managers in applying the use of biological control, this paper will address procedures and techniques that would allow the use of insect biocontrol in existing aquatic plant management programs in a more active manner. Such procedures should, over time, increase the effectiveness of biocontrol and assure that traditional control measures are used in such a manner to minimize impact to biocontrol activities.

## PROCEDURES TO ACCOMPLISH THE ACTIVE USE OF BIOCONTROL

As indicated previously, resource managers use insect biocontrol for the management of aquatic plants primarily through passive efforts. Insect agents are released at a small number of sites, primarily by biocontrol researchers, with only limited participation. No effort is put forth to monitor the sites for establishment, let alone for future increases in the insect populations and their subsequent impact to the plants. To gain the most benefit from biocontrol a more active approach should be taken.

But how does one accomplish the active use of biocontrol and how does it fit in with today's existing management approaches? The existing biocontrol agents should be viewed as resources and should be managed and manipulated for the ultimate goal of achieving a higher degree of effectiveness. There are four steps to an active use of biocontrol including: 1) knowledge, 2) survey, 3) supplement, and 4) integrate. By applying these steps a more effective use of biocontrol, and hence, a higher degree of stress and damage to the target plants, can be achieved.

### Knowledge

The first step to using any new control technology is to gain a thorough comprehension of its basic concepts, an understanding of the different types of control techniques available, and a knowledge of how to apply these control procedures correctly and effectively for each plant species. This is not unlike learning the correct and safest procedures for applying a herbicide or use of a particular type of mechanical harvester.

To use biocontrol effectively and actively you must first understand basic ecological concepts such as population growth. This includes factors affecting population growth and what factors, both abiotic (i.e., weather, climate, temperature, etc.) and biotic (i.e., mortality, reproduction, maturity rates, etc.), that regulate and maintain populations at realistic sizes. In addition, an understanding of how general types of biotic factors interact to keep populations regulated is needed. These include intra-specific biotic factors such as reproductive rates, mortality, and maturity rates, and inter-specific factors, which include competition, i.e., the interaction between the insect agents (i.e., herbivores) and their impact to the target plant.

In addition to these basic ecological concepts, it is necessary to gain more knowledge concerning the agents themselves. One critical aspect is the ability to identify each type of insect agent, its feeding damage and its long-term impact to the plant population. The ability to perform such identifications is complicated by the fact that there are native insects that feed and damage the same target plants. Native species can easily be confused with the introduced insects since they and their feeding damage superficially resemble those of the introduced species. However, with adequate training such identifications can be made easily and accurately.

Several training aids are available for gaining the knowledge needed to use biocontrol more actively. These include training courses offered yearly by the Center of Aquatic Plants in Gainesville, FL, and one offered on demand by the

US Army Corps of Engineers District, Jacksonville or by researchers at WES. In addition, WES researchers have recently developed a CD-ROM entitled the "Noxious and Nuisance Plant Management Information System (PMIS)" that contains identification and damage information on each of the introduced insect agents (Grodowitz et al. 1996). This system also contains information on the use of more traditional management techniques including both chemical and mechanical control technologies. Another CD-ROM (entitled the "Aquatic Plant Information System", APIS) is being developed and will be released in early to mid 1998. This system will contain computer-based identification strategies and information on the insect herbivores, both native and introduced, that feed on 18 commonly encountered aquatic plants. APIS will also contain information on the identification of over 60 aquatic and wetland plant species as well as detailed information on chemical and mechanical control techniques. In addition, the most appropriate person to contact for each plant species is included.

Information on these systems can be obtained by contacting:

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E-MAIL: GRODOWM@MAIL.WES.ARMY.MIL.

In addition to the short courses and computer-based information systems there are also numerous technical reports, journal articles, and videos available from a variety of sources. Center et al. (1990) has a concise listing of the most pertinent literature available on the use of biological control for aquatic plant management and represents a good starting point. WES-published material can be obtained by contacting:

Debra Goodman  
US Army Engineer Waterways Experiment Station  
CEWES-ER-A  
3909 Halls Ferry Road  
Vicksburg, MS 39180  
PHONE: (601) 634-3841

## Survey

The next step in developing an active biocontrol program is to initiate surveys not only of the aquatic plant populations but also of the introduced insect agents. Surveys are important because they reflect past, present, and future population levels of the insects. It is not unusual to find population levels of many of the insect agents to be non-existent or extremely low at certain sites; levels that would not afford any persistent damage and stress to the target plant. Hence, surveys allow resource managers to estimate population abundance/status of the insect agents and make adjustments in population size through supplemental releases, if needed.

The reasons why agent levels decrease vary between insect species. Some factors include a wide range of adverse environmental conditions including extreme temperatures, pro-

longed water level drawdowns, changes in nutritional composition of the plants due to changes in water quality, and wholesale removal of plants. This last factor occurs because of large-scale herbicide applications or mechanical removal. Since the insects, most probably the immature stages, are intimately tied to the plant for food and shelter, any large-scale removal of the plants drastically impacts the insect biological control population (Grodowitz and Pellisier 1998, Grodowitz and Cofrancesco 1990).

Procedures for surveying insect biocontrol agents are quite variable and are dependent on the insect species in question. They are used to determine presence or absence of the insect agents or to provide more detailed information on population size. Surveys that sample for actual population size are more complicated but provide a tremendous amount of useful information. Typically, population size measurements are based on a unit area such as a 1/4 m<sup>2</sup> or on a per plant basis. What complicates the interpretation of the survey results is that insect populations do not remain constant but are continually changing temporally, with such changes related to seasonal and site characteristics as well as the nutritional composition of the plants (Center and Van 1989, Grodowitz et al. 1997). When interpreting survey results, population dynamics as well as the percentage of parous (or fully reproductive individuals) must be taken into account before making decisions regarding the release of additional agents at a given locality (Grodowitz and Cofrancesco 1990).

While the design of surveys for insect biocontrol agents is beyond the scope of the present paper, more detailed information can be obtained from specific insect biocontrol researchers or from the variety of information systems, courses, and journal articles discussed previously under the "Knowledge" section.

## Supplement

The third step in developing an active approach to the use of biological control is based on the information obtained from the surveying methods. If population levels of the agents are low then it may be necessary to release more individuals into the area to supplement or augment the existing population. Recent studies with *Neochetina eichhorniae* (Warner), the mottled waterhyacinth weevil, have shown that supplementing weevil populations significantly increases stress and impact to the plants by quickly raising the population level of the introduced insect species (Center and Jubinsky 1989). Studies conducted with *Hydrellia pakistanae* (Deonier), the Asian leaf-mining fly, have indicated that large, often-repeated releases provide the most damaging effects over the long-term (M. Grodowitz, Vicksburg, MS, unpublished data).

Specific procedures for releasing insect agents vary for each species and can not be discussed in any great detail within the context of this paper. However, certain rules are applicable for all species. First, only utilize high-quality individuals that have not been stressed by the collecting or shipping methods. It is imperative that the insects are kept at relatively constant temperatures, typically below 22°C. They must be shipped at relatively low densities to avoid stress due



to overcrowding. When possible, ensure that the insects are relatively free of disease. Also, continue monitoring the site after the releases have been made to substantiate that the insects have become established, population levels are increasing, and that additional releases are not warranted.

Obtaining insects for release can be time and labor consuming. However, once a suitable site with high insect population levels has been located collecting can proceed quickly. An alternative method is to purchase the insect species from a reputable dealer. Such dealers are quite rare but available in the Florida area. Prices may appear high but considering the cost for locating the insects, travel, collecting time, and handling, the cost is typically quite reasonable.

## Integrate

Integrating biocontrol technology with existing, more traditional, control technologies is probably one of the most important factors in ensuring a viable and active biocontrol program. It makes little sense to use a variety of control methods if one or more interferes or directly impacts the use or effectiveness of another. It has been documented that certain control methods can have an adverse impact on the population size of the insect biocontrol agents (Haag 1986a, Haag 1986b, Grodowitz and Pellessier 1989, and Grodowitz and Cofrancesco 1990). One of these is the use of chemical control methodologies, but any method that removes large quantities of plants relatively quickly would produce the same results. For example, while the chemicals used in the management of aquatic plants are not directly toxic to the biocontrol agents, their use removes, relatively quickly, large quantities of plants from a specific location. Since one or more life stages of the insect agents are directly tied to the plant for food, shelter and, hence survival, such large-scale plant removal kills large numbers of agents, thereby decreasing the population size and ultimate impact to the target plant.

But such impacts can be easily reduced or eliminated. For years many aquatic plant biocontrol researchers (Wright and Center 1984, Center et al. 1990, Grodowitz and Cofrancesco 1990) have recommended leaving unsprayed plants to act as conservation areas or harborage for the biocontrol agents. Such harborage areas ensure the survival of the insect agents and act as a nursery area for further dissemination and spread of the agents when the plants have regrown after the herbicide application. Integration of all existing control measures is simple common sense; utilize all of the available control methods to maximize management benefits and apply them in a manner that minimizes impact to one another.

## SUMMARY

In summary, an active biological control program makes good management sense. It allows the most effective use of a long-term suppression technology, which maximizes control of the target plant. While biological control will never be the ultimate answer for all of the current non-native aquatic and wetland plant problems, it does serve to stress the plants, reduce growth and plant production, and provide long-term suppression, and any reduction in plant biomass is a very positive outcome. But this will only happen if biocontrol is

applied in a logical and active manner that maximizes its effectiveness.

In addition, it is important to gauge the effects of a particular agent based on the understanding that biological control is a long-term suppression technique; a technique that typically does not provide complete elimination of the target plant. The effectiveness of a biological control program must be reviewed in light of the specific impacts caused by an agent and of how they can be utilized to maximum potential. Effectiveness should not be measured in terms of complete eradication or elimination, which is an unusual situation at best. To do so places an unrealistic expectation on the use of biological control and negates the positive aspects of the technology. Biological control is a particularly effective management option especially when considered as a long-term suppression method and should be considered an integral part of a management plan.

## ACKNOWLEDGMENTS

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# Chemical Control Research in the Corps of Engineers

K. D. GETSINGER<sup>1</sup>

## ABSTRACT

Research and development activities in the Corps of Engineers chemical control technology area concentrate on evaluating chemical products and developing application techniques that will improve the management of exotic and nuisance aquatic plants. Current research efforts are focusing on species-selective control of target plants, precision herbicide application techniques, and integration of control strategies with ecological principles. Studies are conducted in controlled-environment chambers, greenhouses, hydraulic flumes, outdoor mesocosms, experimental ponds, and in the field. Cooperators and partners include Federal, state and local agencies, Corps of Engineer Districts, academic institutions, and the private sector. Interactions also occur with Federal and state regulatory agencies. Proven benefits derived from this chemical control research effort include lower herbicide use rates, improved environmental compatibility, and reduced application costs.

**Key words:** aquatic herbicides, aquatic plant management, Eurasian watermilfoil, hydrilla, nuisance exotics, species-selective control.

## INTRODUCTION

When used according to label directions and in a responsible manner, aquatic herbicides are a consistently effective technique for managing nuisance vegetation. In addition, they are economical, environmentally compatible, and safe to use. These traits can make chemical control the method of choice for many aquatic plant management situations. Over the past 10 to 15 years, herbicide use patterns have been maintained, or have increased, in many states infested with exotic aquatic plant species. For example, annual chemical treatments against waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) and hydrilla (*Hydrilla verticillata* Royle) have routinely been used in Florida public waters since 1982 to control 12,000 to 16,000 ha of these plants per annum (Figure 1). In Minnesota, the annual use of selected herbicides in public waters has coincided with the establishment and spread of Eurasian watermilfoil (*Myriophyllum spicatum* L.) in that state, increasing almost 10-fold, from approximately 4,500 kg in 1987 to over 45,000 kg in 1995 (Figure 2). Although herbicides currently play a prominent role in managing aquatic vegetation, improved and innovative uses of these products, new formulations, low-dose application techniques, and species-selective treatment strategies, are directly linked to research and development (R&D) activities. This paper provides an overview of the chemical control R&D efforts, facilities, and capabilities associated with the US

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Army Corps of Engineers (CE) Aquatic Plant Control Research Program (APCRP). Furthermore, a brief summary of the three Federally-funded work units in this technology area will be presented.

### WES CHEMICAL CONTROL TECHNOLOGY TEAM

The CE's APCRP sponsors R&D efforts with aquatic herbicides via the Chemical Control Technology Team (CCTT) at the US Army Engineer Waterways Experiment Station (WES) in Vicksburg, MS. The mission of the CCTT is to evaluate herbicides and develop application techniques for the selective management of exotic and nuisance aquatic vegetation. Practical benefits recently derived from the implementation of this mission include the operational transition to low herbicide use rates, improved environmental compatibility of chemical products and application techniques, improved selectivity to restore native plant communities, and reduced application costs.

Assisting the CCTT in this national R&D effort are several CE Districts such as Baltimore, Jacksonville, Mobile, and Seattle, and Federal Agencies such as the US Department of Agriculture (USDA), the Tennessee Valley Authority (TVA), and the US Bureau of Reclamation (USBR). In addition, state natural resource agencies such as the Alabama Department of Conservation and Natural Resources, the Florida Department of Environmental Protection, the Michigan Department of Environmental Quality, the Minnesota Department of Natural Resources, the South Carolina Department of Natural Resources, Texas Parks and Wildlife Department, and the Washington Department of Ecology cooperate with the CCTT. Finally, academic institutions such as the University of Florida, Purdue University, North Carolina State University, Mississippi State University, and the University of California-Davis, and elements of the private sector such as chemical companies and the Aquatic Ecosystem Restoration Foundation partner with the CCTT. The CCTT also chairs the Federal Aquatic Herbicide Working Group (CE, USBR, USDA, TVA) which interacts closely with

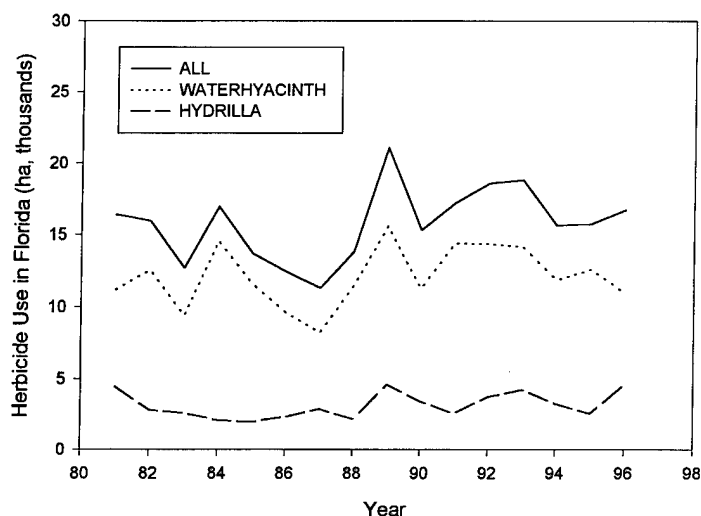


Figure 1. Annual aquatic herbicide use in Florida, 1980-1996. Data provided by the Florida Department of Environmental Protection.

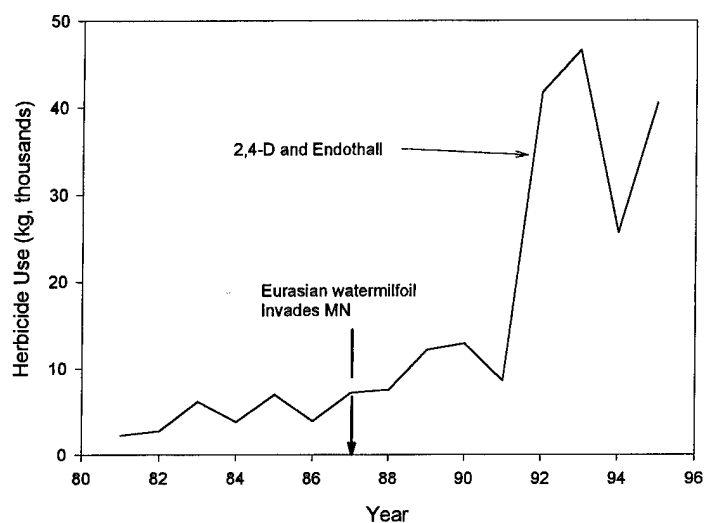


Figure 2. Annual use of the aquatic herbicides 2,4-D and endothall, for controlling Eurasian watermilfoil in Minnesota, 1981-1995. Data provided by the Minnesota Department of Natural Resources.

the US Environmental Protection Agency (USEPA) Office of Pesticide Programs and various state regulatory agencies to discuss and review aquatic pesticide use and registration issues (Getsinger 1995).

The CCTT conducts research at multi-scale levels in facilities that range from laboratory and controlled-environment chambers at WES to greenhouses, large outdoor mesocosms and ponds at the Lewisville Aquatic Ecosystem Research Facility (LAERF) in Texas, culminating with field verification studies conducted in waterways throughout the U.S. By leveraging resources with the sponsors and cooperators noted above, extended lines of research have been possible, leading to major advances in aquatic herbicide technology.

### BENEFITS OF CCTT RESEARCH EFFORTS

One prominent example of the benefits derived from a coordinated and sustained R&D strategy involves the improved use of the herbicide fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone) for controlling hydrilla and Eurasian watermilfoil. In 1986, fluridone received a national aquatic registration (Section 3) from the USEPA, with great promise as a new aquatic plant management tool. Although the product was being applied at the high end of its legal use-rate range (90 to 150  $\mu\text{g/L}$ ) in 1986, target plant efficacy was unpredictable, erratic, and non-selective in many situations, and treatments were costly. By 1993, combined R&D efforts had fine-tuned use rates down to 15 to 20  $\mu\text{g/L}$ , target plant efficacy was predictable and consistent in static water conditions, and inroads into improving efficacy in flowing water were well underway. At present, further research findings have resulted in operational use rates declining to 5-10  $\mu\text{g/L}$  (a > 10-fold decrease from earlier times), while achieving predictable, consistent, species-selective, and low-cost, weed control.

The CCTT has also led a major effort in pursuing a Section 3 aquatic label for the compound triclopyr (3,5,6-trichloro-2-pyridinyl-oxyacetic acid) (Petty et al. 1997a,

1997b, 1997c); which, if registered next year, will be the first aquatic label for a new chemistry in over a decade. In addition, a CCTT-directed R&D coalition evaluated and developed a new superabsorbent polymer carrier for the herbicide endothall (7-oxabicyclo(2.2.1)heptane-2,3-dicarboxylic acid) (Netherland et al. 1998). Once registered, this innovative granular concept will greatly improve the safety factor involved in applicator handling as well as streamline the field application process of this herbicide.

### CHEMICAL CONTROL TECHNOLOGY WORK UNITS

In fiscal year 1997 (FY97), direct allotted funds under the APCRP for chemical control R&D were divided between two work units: (a) Herbicide Delivery Systems; and (b) Species-Selective Use of Aquatic Herbicides and Plant Growth Regulators (PGRs). One additional work unit, Integrated Use of Herbicides and Pathogens for Submersed Plant Control, was also under the direction of the CCTT during FY97. Detailed updates of each work unit can be found in other articles in these proceedings (Nelson et al. 1998, Sprecher et al. 1998, Sisneros et al. 1998).

#### Herbicide Delivery Systems

Information generated in this work unit is used to design systems and/or techniques that can deliver low doses of herbicides over extended periods of time, while providing acceptable target plant control. Environmentally compatible controlled-release carriers, such as polymers, gypsum, proteins, etc., are evaluated for herbicide release rates and efficacy in small-scale systems at the WES. The most promising formulations are further evaluated in large-scale mesocosms and ponds at the LAERF. Results from these studies are used to improve the control of nuisance submersed plants, particularly in areas of high water exchange. Recent work has focused on endothall-polymer formulations and a low-dose metering technology (Netherland and Turner 1995, Sisneros and Turner 1996, Netherland et al. 1998).

#### Species-Selective Use of Aquatic Herbicides and PGRs

While weedy submersed species can be removed using traditional chemical control tactics, these treatments can negatively impact desirable native plant species. However, using chemicals in a species-selective manner (e.g., rate, timing, placement, etc.) can result in the control of target vegetation while enhancing the growth of desirable, beneficial plants by removing weedy competitors. Allowing desirable species to flourish can slow the reinvasion of weedy species and provide improved fish and wildlife habitat. In this way, water bodies plagued with monospecific infestations of exotic plants can be managed to provide a more healthy, diversified, and ecologically-balanced aquatic community. Recent selectivity efforts have evaluated triclopyr (Smart et al. 1995, Getsinger et al. 1997), fluridone (Netherland et al. 1997), and 2,4-D (2,4-dichlorophenoxy acetic acid) (Sprecher et al. 1998).

#### Integrated Use of Herbicides and Pathogens for Submersed Plant Control

One potential method for reducing herbicide rates while maintaining or improving target plant control is to integrate

chemical treatments with known endemic plant-specific pathogens. By combining the strengths of a chemical treatment with a biological organism, the weaknesses of either control technique are often minimized or negated. Potential uses of herbicide/pathogen combinations include: (1) using low rates of herbicide to stress target plants, making them more susceptible to pathogenic attack, and (2) use of pathogens as contact bioherbicides to reduce standing biomass of target plants followed by reduced rates of herbicides to eliminate regrowth or extend efficacy. Selected aquatic herbicide and pathogen combinations are being evaluated for synergistic and/or antagonistic relationships in growth chamber and outdoor mesocosm systems (Netherland and Shearer 1996, Nelson et al. 1998). Promising combinations will be further evaluated in ponds at the LAERF and/or other geographical locations. Results from this work will provide information on the potential of integrating chemical and biological control techniques to improve management of aquatic vegetation.

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*J. Aquat. Plant Manage.* 36: 64-68

# Selective Effects of Aquatic Herbicides on Sago Pondweed

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## ABSTRACT

Three aquatic herbicides effective on the exotic weed Eurasian watermilfoil (*Myriophyllum spicatum* L.)—2,4-D ((2,4-dichlorophenoxy)acetic acid), endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid) and triclopyr ([[(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid)—were evaluated in the laboratory for selective effect or efficacy on the native submersed species, sago pondweed (*Potamogeton pectinatus* L.). For each herbicide, three concentrations in ranges associated with Eurasian watermilfoil or sago pondweed control were applied in static exposures of 24 hr, and plants were monitored for 35 d. Endothall at 0.5, 1 and 2 mg L<sup>-1</sup> significantly reduced final biomass, by  $\geq 72\%$ , confirming that this herbicide will not maintain populations of sago pondweed where it is used to manage Eurasian watermilfoil. Application of the growth regulator-type systemic herbicides at 1, 1.5 and 2 mg L<sup>-1</sup> resulted in no significant reduction in biomass from 2,4-D, but up to 24% reduction with triclopyr. The more selective activity of these compounds towards sago pondweed supports their use for controlling Eurasian watermilfoil in plant communities where it is desirable to maintain the native species. However, in areas where sago pondweed is itself a nuisance plant, endothall gives effective chemical control.

**Key words:** aquatic habitat restoration, aquatic weed control, *Myriophyllum spicatum*, *Potamogeton pectinatus*.

## INTRODUCTION

One of the strengths of herbicide use for vegetation management resides in its potential to provide selective plant control. Some herbicides can eliminate all vegetation while others target only specific groups of plants, and both broad-spectrum and selective herbicides can be manipulated to provide wide flexibility in control by varying application rates and timing. The capacity for selectively eliminating nuisance vegetation without lethal damage to desirable species means that chemical control often provides a precise technique for nuisance and exotic species management in natural ecosystems. In aquatic habitats the effects of available herbicides on exotic or native nuisance weeds are known, and techniques for control of many target species are well described (Van and Conant 1988, Green and Westerdahl 1990, Netherland et al. 1991, Netherland and Getsinger 1992, Netherland et al. 1993). However, the need remains for more information on herbicide effects on native species that enhance the macrophyte communities of aquatic systems.

Documentation of herbicide effects on non-target plants makes it possible to define use rates that result in minimal harm to native plants and allows them to regain their natural balance in the community following weed eradication. The influence of herbicide concentration and exposure time, as well as the timing of application and the physiological condition of plants at treatment (phenology), have been studied in the target plants Eurasian watermilfoil and hydrilla (*Hydrilla verticillata* (L.f.) Royle) (Van and Conant 1988, Green and Westerdahl 1990, Netherland and Getsinger 1992, Netherland and Getsinger 1995, Madsen 1994, Madsen 1997). While herbicide concentrations and exposure requirements are well-described for exotic plants there is much less infor-

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mation available on desirable non-target species such as sago pondweed.

The pondweeds, genus *Potamogeton*, comprise an important monocotyledonous plant family with numerous submersed species of major importance in North American aquatic environments. Sago pondweed is a submersed perennial macrophyte, native to a range of fresh, alkaline, and brackish waters in marshes, lakes, and streams of the United States (Fassett 1957, Godfrey and Wooten 1979). Throughout its natural range the entire plant, particularly its fleshy rhizome and starchy tubers and fruits, provides one of the best food sources for waterfowl<sup>3</sup>, as well as good fish habitat (Godfrey and Wooten 1979). It is frequently recommended for inclusion in plantings to enhance wildlife habitat and to restore lake and reservoir vegetation (Spencer 1987, Smart et al. 1996). The submersed morphology of sago pondweed subjects it to displacement by thick surface canopies produced by non-native weed species such as Eurasian watermilfoil (hereafter "milfoil") or hydrilla (Madsen et al. 1991, Smart et al. 1995). Once these target exotics are eliminated, however, sago pondweed is one of the species that can prevent or slow reinvasion of weeds by colonizing re-opened habitat, having potential to regrow from crowns or rhizomes, or to emerge from seeds or tubers.

The dense growth of sago pondweed often produces problems in the western U.S. where it can choke irrigation canals and significantly impede water flow. The effectiveness of selected herbicides for sago pondweed control has been demonstrated in these high-flow environments (Corbus 1982, Westerdahl and Hall 1983), and there is continued interest in finding minimum rates and effective application techniques for control in irrigation canals (Netherland et al. 1994, Sisneros and Turner 1995). However, there is not much information available on herbicides and use rates that will maintain sago pondweed in those aquatic systems where exotic weeds need to be targeted but where sago pondweed is considered a valuable resource.

Information on herbicide selectivity for sago pondweed under conditions of milfoil control is available from preliminary evaluations of triclopyr and fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone). In outdoor mesocosms with mixed communities of milfoil and four species of native, non-target plants that included sago pondweed, triclopyr applied at 0.5 mg L<sup>-1</sup> for a 12-hr half-life exposure selectively slowed the growth of milfoil and allowed the other species to increase in biomass compared to similar untreated units (Smart et al. 1995). Other treatment combinations of 0.5 or 1.0 mg triclopyr L<sup>-1</sup> with 12- or 24-hr half-life exposures significantly reduced milfoil and increased these native species (Smart et al. 1995). Growth chamber studies with two herbicides indicated their potential selectivity on sago pondweed based on variation in concentration and exposure time (CET). A 24-hr exposure to 2.5 mg triclopyr L<sup>-1</sup> reduced sago pondweed biomass by two-thirds in the month following treatment, while 12 hr at 1 mg L<sup>-1</sup> did not affect biomass (Sprecher 1995). Selective con-

centrations of fluridone were bracketed by 2 and 10 µg L<sup>-1</sup>, as exposure to 10 and 25 µg L<sup>-1</sup> for 60 d decreased sago pondweed biomass to < 2% of untreated controls, while plants at 2 µg L<sup>-1</sup> grew well and underwent normal flowering and seed set in spite of a 24% reduction in biomass (Sprecher 1995). Mesocosm data on individual species showed similar sago pondweed response to differences in fluridone application rates, with recovery from early season 90-d exposures to 5 µg L<sup>-1</sup>, but not to 10 or 20 µg L<sup>-1</sup> (Netherland et al. 1997).

The dipotassium salt of endothall is labeled<sup>4</sup> for control of sago pondweed at a lower rate (1 to 2 mg L<sup>-1</sup> for entire pond or large area treatment, 2 to 3 mg L<sup>-1</sup> for spot or lake margin treatments) than milfoil (1 to 2 and 2 to 3 mg L<sup>-1</sup>, respectively), and selectivity is not expected when using endothall to eradicate milfoil without altering timing or some other application factor to favor the native species. However, there is interest in determining the most efficient CETs for efficacy where sago pondweed is a nuisance plant. To characterize further the selective or control effect on sago pondweed of chemicals used to control milfoil, this study evaluated the response of the pondweed species to less than maximal field application rates of the systemic herbicides 2,4-D and triclopyr, and the contact herbicide endothall.

## MATERIALS AND METHODS

Sago pondweed tubers were acquired from a commercial source (Wildlife Nurseries, Inc., WI) at the end of October 1996, immediately after being harvested from outdoor plantings. They were held refrigerated at 5 ± 2 (standard error: s.e.) °C. After 33 days, tubers were removed from refrigeration and placed in shallow water in light. In three days, shoots had emerged up to 5 cm. Four sprouted tubers were then planted per each glass beaker holding 250 ml of lake sediment previously amended with nitrogen at 12.5 mg NH<sub>4</sub>Cl L<sup>-1</sup>. Ten planted beakers were placed in each of 52 aquaria holding 49 L of simulated hard water (Smart and Barko 1984). Aquaria were held in a controlled environment chamber maintained at 24 ± 2 °C under illumination at 412.7 ± 11.7 µE m<sup>-2</sup> sec<sup>-1</sup> for 14L:10D cycles, with constant aeration. Simulated hard water was refreshed via flow-through exchange three times a week. After 25 days plant shoots had reached the top of the water column (66 cm) and were healthy, flowering and setting seed. At 28 days of growth, immediately prior to herbicide treatment, all plant material from three randomly-selected aquaria was harvested and dried for 48 hr at 70 °C to determine initial average dry weight (DW) biomass per treatment unit.

Herbicides, formulations, and treatment CETs used in the study are shown in Table 1, along with labeled rates and treatment guidelines for milfoil established in previous laboratory CET studies. These soluble concentrate formulations were applied to aquaria as stock solutions diluted with distilled water. Treatment concentrations were calculated based on active ingredient (ai), or acid equivalent (ae) of herbicide compound. Each treatment was replicated in three randomly-assigned aquaria, and three aquaria were left

<sup>3</sup>Eggers, S. D. and D. M. Reed. 1997. Wetland Plants and Plant Communities of Minnesota and Wisconsin. 2nd edition. U.S. Army Corps of Engineers, St. Paul District, St. Paul, MN, 263 pp.

<sup>4</sup>Elf Atochem. 1995. Aquathol® K aquatic herbicide label. Elf Atochem North America, Inc. Philadelphia, PA, 4 pp.

TABLE 1. FORMULATIONS AND CONCENTRATIONS/EXPOSURE TIMES (CETs) OF THREE HERBICIDE TREATMENTS APPLIED TO ASSESS SELECTIVITY ON SAGO PONDWEED (SPW). RECOMMENDED RATES FROM FORMULATION LABELS (MG L<sup>-1</sup> WHERE GIVEN), AND RESULTS FROM PREVIOUS CET STUDIES FOR CONTROL OF EURASIAN WATERMILFOIL (EWM), INCLUDED FOR COMPARISON.

Herbicide/Formulation	Concentrations/Exposure Times	Recommended Rates from Labels and Results from Previous CET Studies <sup>1</sup>
2,4-D WEEDAR 64 38.9% ae <sup>2</sup>	1.0 mg L <sup>-1</sup> 24 hr 1.5 mg L <sup>-1</sup> 24 hr 2.0 mg L <sup>-1</sup> 24 hr	EWM: 10.6 to 42.6 kg ae ha <sup>-1</sup>  Green and Westerdahl 1990 2 mg ae L <sup>-1</sup> for 24 hr → control Netherland et al. 1991 1 mg ae L <sup>-1</sup> /24 hr → 75% control 6 WAT 2 mg ae L <sup>-1</sup> /24 hr → ≥ 75% control 6 WAT
Endothall AQUATHOL K 40.3% ai	0.5 mg L <sup>-1</sup> 24 hr 1.0 mg L <sup>-1</sup> 24 hr 2.0 mg L <sup>-1</sup> 24 hr	EWM: 2 to 3 mg L <sup>-1</sup> SPW: 1 to 2 mg L <sup>-1</sup>  Netherland et al. 1991 EWM: 2 mg ai L <sup>-1</sup> /24 hr → ≥ 85% control 6 WAT
Triclopyr GARLON 3A 31.8% ae	1.0 mg L <sup>-1</sup> 24 hr 1.5 mg L <sup>-1</sup> 24 hr 2.0 mg L <sup>-1</sup> 24 hr	EWM: 1 to 2.5 mg L <sup>-1</sup> (proposed label)  Netherland and Getsinger 1992 1.5 to 2.5 mg ae L <sup>-1</sup> for 24 hr → 85% control

<sup>1</sup>See current herbicide label for complete application recommendations; see Literature Cited for full references.

<sup>2</sup>Abbreviations: ae, acid equivalent; ai, active ingredient; WAT, weeks after treatment.

untreated as reference units. Immediately following treatment exposures, aquaria were drained and re-filled three times with untreated culture medium, in order to remove all herbicide residues. Plants were then maintained for an additional four and a half weeks under the same growing conditions provided pretreatment, and were monitored via visual observations of physical condition on a weekly basis. At 35 days after treatment (DAT), viable plant tissue remaining in treated aquaria and the untreated reference units was harvested and dried to determine final DW biomass.

Statistical comparisons of final DW biomass data were made using SigmaStat (Jandel 1992). Within each herbicide, ANOVA were carried out on the three treatment levels and the untreated reference, and the Student-Newman-Keuls pairwise multiple comparison method was used to show significant differences among concentrations ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

At time of herbicide application, plant biomass had reached an average of  $11.8 \pm 0.76$  g DW per treatment unit (aquarium), or 188.8 g DW m<sup>-2</sup>, comparable to mid-season field production of sago pondweed in natural systems and irrigation flumes (Davis and Carey 1981, Madsen and Adams 1989, Sand-Jensen et al. 1989, Sisneros and Turner 1995). With 25 d growth, the original tubers had produced roots and healthy rhizome systems, although these tissues were not included in top growth biomass measurement. Treatment of actively growing plants at the flowering stage also represents response in sago pondweed populations that have reached nuisance proportions.

By 4 DAT, there were marked differences among treatments. Plant canopies in aquaria treated with endothall already had a brownish appearance; those treated with triclo-

pyr and 2,4-D remained bright green. Most damage was seen at the highest endothall rate, 2 mg L<sup>-1</sup> for 24 hr, where the pondweed had already lost tissue integrity and was being colonized by algae, suggesting cell rupture and leakage of nutrients. Distinct epinastic curling of leaf, tips, shoot apices, and flower stalks had occurred in treatments with the auxin-type herbicides 2,4-D and triclopyr at this time.

At 11 DAT, triclopyr treatments showed the least damage and effects, with epinastic curling evident only in scattered leaf tips; otherwise stems and tissue were firm and green. 2,4-D had produced slight chlorosis and leaf-tip curling. Plants treated with 1 or 2 mg endothall L<sup>-1</sup> showed symptoms that included softening of leaf and stem tissue, initial collapse of the plant canopy, stem chlorosis, dark green "water-soaked" areas in leaves suggesting disintegration of tissue, and epiphytic algal colonization. Plants in untreated reference aquaria remained healthy and bright green, with resilient stems.

A week later (18 DAT), differences among herbicides had become more pronounced. The higher rates of endothall had produced water-soaked, decomposing tissue that was heavily colonized by algae. Plants subject to the other herbicide treatments, as well as the untreated references, remained in good condition, although some apical curvature was still present in upper shoots of triclopyr-treated plants.

At 31 DAT, most plants in the endothall-treated units consisted of leafless stems, but in several cases a few short new shoots had emerged from crowns. Full canopies and active growth, with flowering and seed set, had been maintained in all triclopyr and 2,4-D-treated units. Harvest data at 35 DAT indicated that DW biomass had doubled in untreated aquaria during the month following herbicide application, increasing to  $23.6 \pm 1.91$  g. Effects of treatment levels within individual herbicides varied significantly for endothall and triclopyr in comparison to this untreated material (Figure 1).

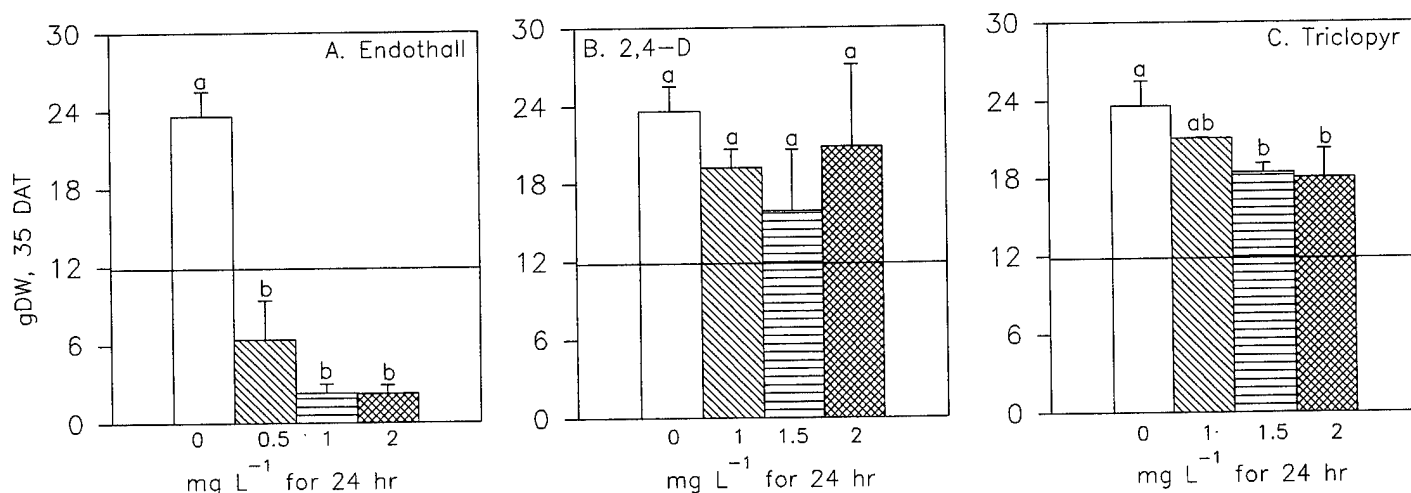


Figure 1. Biomass in grams dry weight (g DW) at 35 days after treatment (DAT) of sago pondweed with various CETs of the contact herbicide, A. endothall, or the systemics, B. 2,4-D, and C. triclopyr. Untreated reference shown with each treatment. Line indicates pretreatment biomass,  $11.8 \pm 0.76$ . Bars above histograms represent standard errors of the mean,  $N = 3$ . Letters indicate significant treatment differences,  $p < 0.05$ .

The systemic herbicides 2,4-D and triclopyr, both with growth regulator modes of action that generally target dicot and broadleaf monocot species, had significantly less effect on sago pondweed biomass than the contact herbicide endothall. Although the systemic compounds produced characteristic epinasty, related to overgrowth of meristematic cells, plants retained vigor, and significant biomass reduction was produced only by the higher rates of triclopyr (Figure 1). While Westerdahl and Hall (1983) showed that 2,4-D reduced sago pondweed biomass by half with 0.10 to 0.25 mg L<sup>-1</sup>, this effect was produced following 11 weeks of constant exposure to these low concentrations. Since a more-readily achieved exposure time of 24 hr to concentrations of 1 and 2 mg 2,4-D L<sup>-1</sup> maintained  $\geq 75\%$  reduction in milfoil biomass through 6 weeks after treatment (WAT) (Green and Westerdahl 1990, Netherland et al. 1991), these CETs will effectively target milfoil while retaining sago pondweed in an infested environment.

The lowest triclopyr concentration did not reduce biomass significantly, and this result can be compared to the lack of effect following a 12-hr exposure to 1 mg triclopyr L<sup>-1</sup> previously seen in sago pondweed (Sprecher 1995). Although treatment with 1.5 or 2 mg L<sup>-1</sup> significantly decreased biomass production by  $\geq 22\%$ , plants maintained full canopies and underwent normal life-cycles, flowering and setting seed. Exposures of 24 hr at these concentrations effectively control milfoil, eliminating 85% of biomass (Table 1; Netherland and Getsinger 1992). However, since Sprecher (1995) showed that an exposure of 24 hr to 2.5 mg triclopyr L<sup>-1</sup> reduced sago pondweed biomass by two-thirds, CETs of 1.5 to 2 mg triclopyr L<sup>-1</sup> for 24 hr are indicated for targeting milfoil where subsequent rapid recovery of sago pondweed populations from plants is desired.

Results from both growth regulator herbicides indicate that they are able to eliminate or greatly reduce the presence of milfoil in the field at rates that allow for rapid recovery and recolonization by sago pondweed. With treatment early in the year, at a growth stage prior to that evaluated here, milfoil is expected to be readily controlled at lower rates with

subsequent regrowth of this pondweed from tubers and rhizomes as well as plants.

The contact herbicide endothall reduced biomass below pretreatment levels, to  $\leq 28\%$  of final untreated biomass, and use of this compound to eliminate the target weed milfoil is not recommended where sago pondweed is to be maintained. This is consistent with label recommendations (Table 1) that indicate that this pondweed is more sensitive than the target weed. There were no significant differences among endothall treatments, indicating that where sago pondweed is to be controlled, concentration  $\geq 0.5 < 1$  mg L<sup>-1</sup> may give adequate efficacy with a 24-hr exposure, particularly if early-season treatment of younger plants is possible.

These various responses in sago pondweed to herbicide application indicate the range of vegetation management options provided by chemicals. The selective effect of the growth regulator herbicides with their auxin-like activity make them suitable for operational application on milfoil in habitats where this native pondweed is to be maintained. As a narrow-leaf monocot, sago pondweed is more similar to hydrilla in not being seriously affected by 2,4-D or triclopyr. In this study, treatment of actively growing plants at the flowering stage represents response in sago pondweed populations that have reached nuisance proportions, or a "worse-case" scenario, where this species is the target weed. Control of sago pondweed by the contact herbicide endothall may be made more efficient by timing lower treatment rates to earlier stages of growth.

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# Low-Dose Metering of Endothall for Aquatic Plant Control in Flowing Water

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## ABSTRACT

Endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid) applied as Aquathol K<sup>®</sup> at a target rate of 0.4 mg/L for 84 hr was evaluated in two high-flow canals located in Idaho and Colorado in the late summer of 1996 against the nuisance species sago pondweed (*Potamogeton pectinatus* L.). A prototype metering pump was developed by U.S. Bureau of Reclamation personnel to deliver a constant low dose of herbicide in response to the variable flow rates often experienced in these canal systems. Residue analyses indicated that target herbicide rates were closely approximated through 84 hr in the Idaho study, with a resultant 97% reduction of sago pondweed biomass along the 1.6-km treatment site at 28 days posttreatment. In contrast, biomass at untreated control sites remained near pretreatment levels of 120 g dry weight/m<sup>2</sup>. Target endothall concentrations were maintained through 55 hr at the Colorado site, followed by an unexplained drop in endothall levels to one half the target concentration through 84 hr. Biomass reductions of 60 to 98% were recorded along the 5.3 km treatment site by 17 days posttreatment. Results demonstrated that the low-dose, extended exposure concept for endothall treatment was efficacious and logistically feasible. In addition, the newly developed metering pump may have great utility for herbicide application in high-flow environments of the western United States.

**Key words:** aquathol, aquatic herbicide, *Potamogeton pectinatus*, Sago pondweed, aquatic weed control.

## INTRODUCTION

Aquatic plants can severely restrict water flow in delivery channels carrying agricultural, industrial, municipal, and recreational waters. In the western United States, traditional chemical control techniques have included the use of rapid acting contact biocides such as acrolein (2-propenal) and xylene (Anderson 1990); however, increased concern over toxicity of these products to fish and wildlife in waters managed by the U.S. Bureau of Reclamation (Reclamation) has led to an interest in alternative chemical control methods. Although use of other herbicides may lessen concerns over toxicity, traditional slug or surface applications of many of these compounds may not be efficacious in canals due to rapidly dissipating concentrations and a concomitant lack of

exposure period (Hansen et al. 1983). Concentration and exposure time studies conducted in the laboratory and in large outdoor hydraulic flumes have demonstrated that delivery of the herbicide endothall at concentrations of 8 to 10% (0.4 to 0.5 mg/L) of the maximum label rate (5.0 mg/L) over an extended exposure period can be efficacious for control of nuisance aquatic vegetation (Netherland et al. 1991, Netherland et al. 1994). Endothall applied as the Aquathol K formulation has low potential for fish and wildlife toxicity even at the maximum use rate of 5.0 mg/L (Elf Atochem<sup>®</sup>). Based on this information, a pilot study was conducted in Idaho in 1994 to determine the feasibility and potential logistical problems associated with applying low doses of endothall (applied as Aquathol K) for sago pondweed control in a western irrigation system<sup>4</sup>. The ability of sago pondweed to thrive in high-flow environments and form a surface canopy that impedes water flow, makes it a particularly troublesome plant in the western United States. A good review of the ecology, biology and early methods used for control of sago pondweed in the Pacific Northwest is provided by Yeo (1965).

Pilot study results<sup>4</sup> were marginal in terms of significant biomass reduction of sago pondweed. Problems with responding to the highly fluctuating canal flow rates by continuous manual adjustment of the metering equipment prevented maintenance of the target endothall concentration of 0.4 mg/L for 96 hr. Despite the problems with achieving target rates, the endothall treatments did result in the removal of the sago pondweed canopy within 1 week after treatment, and project managers were generally pleased with the efficacy results through 42 days posttreatment. The conclusions of this study were that the low dose extended exposure concept was feasible and could be efficacious; however, significant improvement in the delivery system would be required to make this strategy practical. In response to problems encountered during the 1994 study, Reclamation developed an automated metering system that maintains the desired herbicide concentration in linear-flow systems with fluctuating flow rates. This system was designed for use at remote sites, is totally portable, and can be operated using solar power. This new pump will significantly reduce manpower requirements and the lag time associated with manually

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<sup>3</sup>Elf Atochem, 1992. Review of the effects of endothall products on aquatic ecosystems

<sup>4</sup>Sisneros, D. and E. G. Turner. 1996. Reduced rate endothall application for controlling sago pondweed in high-flow environments: Summary of 1994 study. Technical Memorandum 8220-96-12. U.S. Department of Interior, Bureau of Reclamation. 5 pp.

adjusting metering pumps following flow rate measurements.

Two metering studies using low concentrations and extended exposure periods were conducted by Reclamation to evaluate the automated metering system and to determine the efficacy of metering low concentrations of the herbicide endothall (i.e. at rates approaching 8% of the maximum label rate) to control sago pondweed in flowing water.

## MATERIALS AND METHODS

### Idaho Site

A field site was selected in Reclamation's Pacific Northwest Region, in the Minodoka Project on land operated and maintained by the North Side Canal Company, Jerome, ID. Treatments were conducted on the S-19 canal from August 6 to August 9, 1996. The portion of the S-19 canal used for this study carries return flow irrigation water (waste water) and is approximately 10 miles southwest of Wendall, ID.

This earth lined canal is approximately 3 to 4 m wide and 0.7 to 1.0 m deep. Flows in the system during the study ranged from 145 to 285 l/sec (5.1 cubic feet per second (cfs) to 10 cfs) with linear velocities of approximately 0.5 m per second. The length of canal treated by the upstream injection of herbicide was approximately 1.7 km. Below this point, dilution from the inflow of the W-9 canal further diluted endothall concentrations. The combined flows from both canals continued for 3.2 km to a pond of 1.3 ha. Here additional flows from another canal further diluted any treated water prior to discharge into the Snake River, approximately 2.4 km downstream of the pond.

Four plant biomass sampling sites (30.5 m long) were located within the 1.7 km treatment area to determine herbicide efficacy. One untreated biomass sampling site was selected 200 m above the application point. Sampling sites were selected in areas with high densities of plants (visual observations) between the application site and sampling site 5 at 1.2 km downstream.

Nine random shoot biomass samples were taken from each of the biomass sampling sites using a 0.25 m square frame prior to treatment and at 28 days after treatment (DAT). Biomass samples were then sorted to species and dried at 100 C for 24 hr to a constant weight. The major weed species was sago pondweed with small amounts of *Alisma* sp. L. (water plantain). Shoot biomass data from each sample site were subjected to analysis of variance (ANOVA) and means compared to the untreated controls using Dunnett's test at  $\alpha = 0.05$ . In addition, t-tests ( $\alpha = 0.05$ ) were used to compare pretreatment biomass to biomass at 28 DAT within each sampling site.

Six water sampling sites were established for endothall residues downstream of the herbicide application site. Untreated control water samples were taken immediately upstream of the herbicide application site. Water samples were collected at 200, 411, 500, 586, and 1600 m from the application site and from Lemon Power Plant (3800 m), which received water from the treated canal for use in power production prior to discharge into the Snake River, ID. Duplicate water samples were collected from each of the six water sampling sites at pretreatment, 7, 12, 28, 36, 48, 60, 72

and 84 hr posttreatment. Water samples were collected from mid-depth, and frozen as soon as possible to reduce herbicide degradation prior to analysis. To date, residue analyses have been completed for sites 2 (411 m) and 4 (586 m), the untreated control, and Lemon Power Plant.

The dipotassium salt of endothall (Aquathol K) was applied to the S19 canal at a rate of 0.4 mg/L for 84 hr using an automated delivery system which is currently patent pending. This system is portable, solar powered, and allows real-time adjustment of delivery of herbicide to the canals.

### Colorado Site

A field site was also selected in Reclamation's Great Plains Region on land operated and maintained by the Farmers Independent Ditch Company (FIDCO), Gilcrest, CO. The FIDCO canal is earth lined and approximately 3.1 m wide and 0.7 m deep. Flows measured at check structures and gauging stations ranged from 74 to 327 l/sec (2.22 cfs to 9.81 cfs) with linear velocities of 0.5 m/sec. The length of the canal which was treated was approximately 5.3 km and this canal was subsequently diverted to a 1.3 ha augmentation pond for recharging ground water during the study.

Five biomass sampling sites (30.5 m long) were located within the 5.3 km area to determine endothall's efficacy on sago pondweed. In addition, one untreated control biomass sampling site was located upstream of the application site. Biomass sampling sites were selected in areas with high densities of plants (visual observations) between the application site and 5.3 km downstream.

Nine random shoot biomass samples were taken from each of the biomass sampling sites using a 0.25 m square frame prior to treatment and at 17 DAT. Biomass samples were then sorted to species and dried at 70 C for 48 hr to a constant weight. The major aquatic weed species was sago pondweed. Shoot biomass data from each sample site were subjected to analysis of variance (ANOVA) and means compared to the untreated controls using Dunnett's test at  $\alpha = 0.05$ . In addition, t-tests ( $\alpha = 0.05$ ) were used to compare pretreatment biomass to biomass at 17 DAT within each sampling site.

Six water sampling sites were established for endothall residues at 800, 1600, 2400, 4000, and 5300 m downstream of the herbicide application site. Untreated control water samples were taken immediately upstream of the herbicide application site in the control biomass sampling site. Duplicate water samples were collected from each of the six water sampling sites at pretreatment, 6, 12, 24, 36, 48, 55, 72, 84 and 96 hr posttreatment. Water samples were collected from mid-depth, and frozen as soon as possible to reduce herbicide degradation for analysis.

Endothall applied as Aquathol K was metered in to the FIDCO canal from September 23, 1996 to September 28, 1996, at a target rate of 0.4 mg/L for 96 hr using the automated delivery system described above.

## RESULTS AND DISCUSSION

### Idaho Site

Residue analyses indicated that the projected target rate of 0.4 mg/L (endothall) for 84 hr was closely approximated

(Figure 1). Endothall residues for the treatment sites in the canal were approximately 0.30 mg/L or greater and were maintained for 84 continuous hours. Concentrations of approximately 0.40 mg/L were noted at 7, 24, and 72 hr posttreatment while residues of 0.30 or greater were noted at the 12, 36, 48, 60 and 84 hr posttreatment sampling. Some loss of endothall is to be expected due to degradation, sorption, and uptake, and the maintenance of rates near the target concentration was considered successful. As metering was discontinued, residues in the treatment sites fell to near 0 mg/L by 96 hr posttreatment. Concentration of less than 0.2 mg/L (the acceptable residue level for drinking water of 0.2 mg/L) were noted in Lemon Power Plant water which eventually flows into the Snake River. Residues in the untreated control were near 0 mg/L throughout the sample period.

Following endothall treatment, sago pondweed and water plantain were significantly reduced by the 28 day posttreatment sampling (Figure 1). Pretreatment biomass ranged from 30 to 150 g dry weight/m<sup>2</sup> whereas at 28 DAT biomass ranged from 120 g dry weight/m<sup>2</sup> for the untreated control site to 0 to 2 g dry weight/m<sup>2</sup> for the treated sites. Visual assessments by Northside Canal operational personnel suggested that sago pondweed recovered to about 30% of the

pretreatment levels during the remainder of the growing season, but was not considered to be at problematic levels.

## Colorado Site

Residue analyses indicated that endothall concentrations ranged from 0.27 to 0.35 mg/L for 55 hr through the entire 5.3-km length of canal (Figure 2). After the 55-hr sampling, residues at all sampling sites dissipated to approximately 0.2 mg/L for the duration of the study (84 hr). Some discrepancies in residue analysis were noted in duplicate water samples which may account for the variability observed at 36, 48 and 55 hr. While the inability to achieve exactly the target concentration of 0.4 mg/L was not surprising, due to previously described factors, the decrease in residues at 55 hr remains unexplained. Based on the loss of residue after 55 hr, it must be assumed that either flow rates increased without a concomitant increase in delivery rate, or the delivery rate slowed without a decrease in flow. Regardless of these possibilities, this represented the initial phase of testing of the new metering pump and results were generally quite positive. The pump is still in the research phase and will require some development and refinement by Reclamation personnel prior to routine operational use in the field.

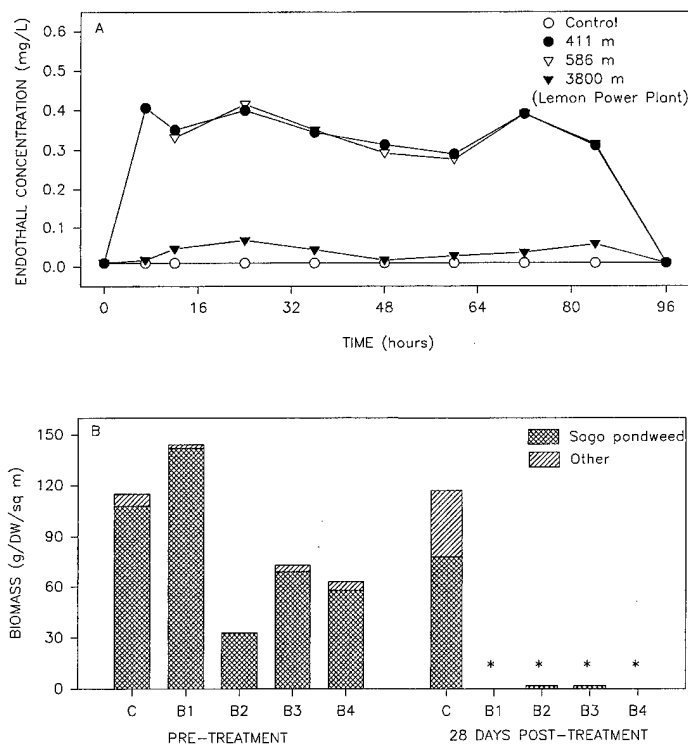


Figure 1. (A) Endothall residues at four sites in the S19 Canal in Idaho following metered injection at a target rate of 0.4 mg/L for 84 hr. Each value represents the mean of duplicate samples. (B). Sago pondweed biomass at pretreatment and 28 days posttreatment collected at 5 sites from the S19 Canal in Idaho. Each bar represents the mean of 9 samples. Asterisks above the bars at 28 days posttreatment indicate that biomass at sampling sites B1-B4 was significantly different from the untreated control at 28 DAT (Dunnett's test at  $\alpha = 0.05$ ) and was also significantly different from pretreatment biomass values (t-test,  $\alpha = 0.05$ ).

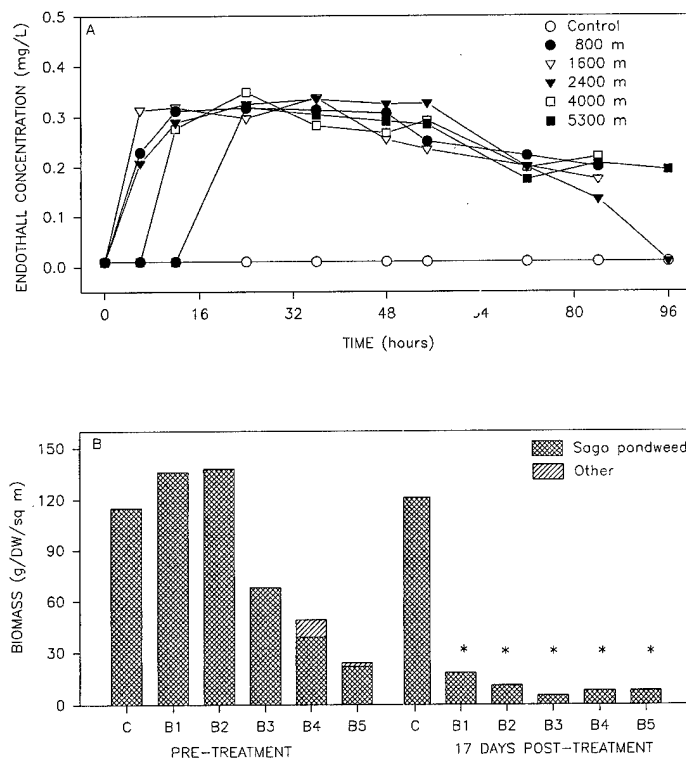


Figure 2. (A) Endothall residues at six sites in the FIDCO canal in Colorado following metered injection at a target rate of 0.4 mg/L for 84 hr. Each value represents the average of duplicate samples. (B). Sago pondweed biomass at pretreatment and 17 days posttreatment collected at 6 sites from the FIDCO canal in Colorado. Each bar represents the average of 9 samples. Asterisks above the bars at 28 days posttreatment indicate that biomass at sampling sites B1-B4 was significantly different from the untreated control at 28 DAT (Dunnett's test at  $\alpha = 0.05$ ) and was also significantly different from pretreatment biomass values (t-test,  $\alpha = 0.05$ ).

Despite lower than targeted endothall concentrations, sago pondweed was significantly reduced by the 17 DAT sampling (Figure 2). Pretreatment biomass ranged from approximately 25 to 137 g dry weight/m<sup>2</sup>, whereas biomass at 17 DAT was 115 g dry weight/m<sup>2</sup> for the untreated control site and ranged from 4 to 20 g dry weight/m<sup>2</sup> for the treatment sites. The late fall treatment, and the fact that these canals were subsequently dewatered, precluded further evaluation of sago pondweed regrowth.

Both the Idaho and Colorado studies demonstrated that delivery of low concentrations of Aquathol K (0.3 to 0.4 mg endothall/L) for 84 continuous hours can effectively control sago pondweed. Based on laboratory information per concentration and exposure time relationships, surface applications of higher rates of endothall (3.0 to 5.0 mg/L) along the length of these canals would not have been effective due to inadequate exposure periods. In addition, off-target movement of these higher residue levels could cause concern where fish, wildlife and potable water are issues. Current irrigation restrictions on the Aquathol K product (7 day restriction at the rates applied) would have to be substantially reduced for the low concentration/extended exposure strategy to find widespread use in Reclamation managed waters; however, dissipation studies to generate the type of data required for label changes are currently planned by the manufacturer.

The delivery pump developed by Reclamation significantly reduced manpower requirements compared to earlier conventional applications and required no maintenance or calibration during the study. Due to the development of this pump, the strategy of delivering low doses of herbicide over time is considered to be quite feasible from an operational standpoint. Although still in the development stage, this pump has excellent potential for precision delivery of herbi-

cides in linear flow systems. The ability to deliver such defined doses should spur future concentration and exposure time studies with several classes of herbicides to determine the minimum rates and exposures necessary for control of a given nuisance species.

## ACKNOWLEDGMENTS

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# Mesocosm Evaluation of Integrated Fluridone-Fungal Pathogen Treatment on Four Submersed Plants

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## ABSTRACT

An outdoor mesocosm study was conducted to evaluate the efficacy and selectivity of the herbicide fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone) and the fungal pathogen *Mycoleptodiscus terrestris* (Gerd.) Ostazeski (*Mt*), applied alone and in combination with one another, against hydrilla (*Hydrilla verticillata* (L. f.) Royle), Eurasian watermilfoil (*Myriophyllum spicatum* L.), American pondweed (*Potamogeton nodosus* Poiret), and vallisneria (*Vallisneria spiralis* L.). Treatments included 5 µg/L fluridone, 100 and 200 colony forming units (CFU) per ml of *Mt*, integrated treatments of 5 µg/L fluridone + 100 or 200 CFU/ml *Mt*, and untreated controls. Treatment with either fluridone or 200 CFU/ml *Mt* alone was sufficient to reduce hydrilla growth by 40% 84 days after treatment (DAT); however, the combined application of *Mt* plus fluridone reduced biomass by 93% compared with untreated plants. Eurasian watermilfoil biomass was not affected by *Mt* alone and was equally inhibited with treatment of fluridone or fluridone with *Mt* (75% reduction at 84 DAT). Treatments did not inhibit biomass production of American pondweed or vallisneria. With the exception of American pondweed, all treatments that included fluridone significantly reduced total chlorophyll. Results show that integrating a low dose of fluridone (5 µg/L) with *Mt* can effectively and selectively reduce hydrilla biomass with minimal effect to non-target plant species such as vallisneria and American pondweed, but may not improve control of Eurasian watermilfoil over fluridone alone.

**Key words:** *Hydrilla verticillata*, integrated control, fungal pathogen, *Mycoleptodiscus terrestris*, *Myriophyllum spicatum*, *Potamogeton nodosus*, *Vallisneria spiralis*.

## INTRODUCTION

Several investigators have reported that the efficacy of some plant pathogens can be enhanced by integration with chemical herbicides (Charudattan 1986, Hoagland 1996, Netherland and Shearer 1996, Rayachhetry and Elliot 1997). In a recent review, Hoagland (1996) stated that although

many pathogens have been characterized as bioherbicides, most lack sufficient aggressiveness to overcome weed defense mechanisms to achieve adequate control. However, some herbicides and plant growth regulators can act to weaken natural plant defense systems, rendering them more susceptible to pathogen attack (Hoagland 1996). Interactions between control agents may be antagonistic, synergistic, or additive, with additive and synergistic effects desirable for maximizing weed control. The potential advantages for implementing an integrated management strategy include: increased efficacy, reduced herbicide and pathogen levels required for weed control, expanded pathogen host range, and a more economically and environmentally acceptable method of nuisance plant management (Charudattan 1986, Hoagland 1996).

Using an integrated approach for managing the aquatic weeds waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) and Eurasian watermilfoil, has been investigated by others (Charudattan 1986, Sorsa et al. 1988). Recently, Netherland and Shearer (1996) demonstrated that combining low doses of the systemic herbicide, fluridone, with a fungal pathogen, *Mt*, was effective for controlling the nuisance exotic plant hydrilla, in growth chamber trials. Applying a sublethal dose of fluridone (2 µg/L) with *Mt* at rates of 100 and 200 CFU/ml reduced hydrilla biomass by >90% and was more efficacious than applying either control agent alone. The integrated treatment provided the benefits of rapid biomass reduction exhibited by *Mt* and long-term prevention of hydrilla regrowth provided by fluridone. In addition, integrated treatments reduced fluridone exposure requirements by approximately 50 days, which may broaden the use of this herbicide in aquatic environments where high water exchange has limited its use in the past. Fluridone generally requires a contact time of 60-90 days to achieve satisfactory hydrilla control and thus has limited use in aquatic systems where high water exchange precludes long chemical-plant exposure periods (Netherland et al. 1993, Netherland and Getsinger 1995).

Herbicide selectivity can often be achieved by applying lower than recommended dosages to sensitive vegetation. Selective removal of a nuisance plant species without damaging non-target plants is a desirable goal for many aquatic plant management situations. One advantage that may result from integrating fluridone with *Mt* is that lowering the fluridone concentration may allow increased species selectivity. Netherland et al. (1997) demonstrated in a mesocosm study that 60- and 90-day exposures of 5 µg/L fluridone were suffi-

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<sup>2</sup>Lilly Research Laboratory. 1980. Method AM-AA-CA-R005-AC-755: determination of fluridone in water by direct injection high pressure liquid chromatography. Eli Lilly and Co., Greenfield, IN. 4 pp.

cient to significantly reduce Eurasian watermilfoil biomass with no effect on biomass production of non-target species (elodea (*Elodea canadensis* Mich.), American pondweed, sago pondweed (*Potamogeton pectinatus* L.), and vallisneria), whereas higher fluridone rates (10 µg/L) severely damaged all non-target species. Thus the potential exists to control the growth of noxious species with reduced rates of fluridone, without affecting desirable, native species.

The objectives of this study were: to verify laboratory efficacy of integrating fluridone with *Mt* for control of hydrilla, the target weed, under outdoor growing conditions; and to determine the specificity of fluridone-*Mt* treatment on other submersed plant species.

## MATERIALS AND METHODS

This experiment was conducted in an outdoor mesocosm system at the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX, that consists of large tanks (1.4 m tall by 2.6 m in diameter) which hold approximately 6500 L of water. Each tank was individually plumbed to regulate water flow as needed and was equipped with air flow for water circulation. Further description of this mesocosm system can be found in Dick et al. (1997).

For this study, each mesocosm tank (18 total) was divided into four equal sections with netting to accommodate each of the four plant species. The netting allowed water flow between the divided areas but restricted plant growth to each section. Plants were grown in plastic pots (19.7 cm tall by 19.7 cm in diameter) filled with nutrient-enriched soil (1 Woodace briquette (14-3-3) plus 10 g ammonium sulfate per pot). Nine pots of each plant species (3 plants per pot) were placed in each tank section. Hydrilla (dioecious biotype) and Eurasian watermilfoil were propagated from 10-cm apical cuttings and planted 4 to 5 cm into the soil. American pondweed and vallisneria were initiated from pre-germinated tubers placed 4 to 5 cm into the soil. All plants and tubers used in this study were collected from pond-grown cultures at the LAERF. Plants were allowed to establish in the mesocosm tanks for 2 months prior to herbicide-pathogen treatment. At the time of treatment, hydrilla and Eurasian watermilfoil had grown to the water surface, American pondweed had formed a surface canopy of floating leaves, and vallisneria was well established.

Treatments were applied on 19 June 1996 and included 5 µg/L fluridone, 100 and 200 CFU/ml of *Mt*, integrated treatments of 5 µg/L fluridone + 100 or 200 CFU/ml *Mt*, and untreated controls. Fluridone stock solutions were prepared from the liquid commercial formulation Sonar® AS (479 grams active ingredient per liter). *Mt* (isolated from hydrilla in TX) was applied as a thick slurry of live fungal mycelium. The *Mt* inoculum was prepared as described by Shearer (1996). Both fluridone and *Mt* were applied by pouring the chemical solution and the mycelial suspension evenly over the water surface. Integrated treatments were applied simultaneously to designated tanks.

Plant biomass was harvested at 21, 42, and 84 days after treatment (DAT). At each harvest, 3 randomly selected pots of each plant species were removed from each mesocosm tank. Aboveground biomass was clipped at the sediment surface, washed to remove algae and debris, and dried to a con-

stant weight at 60 °C. Plant biomass was recorded as g dry weight/pot.

Fresh tissue samples (4 samples per plant species per tank) were collected pretreatment and at each posttreatment harvest for chlorophyll analysis. The tissue selected for this procedure varied for each plant species and included 4-cm stem apices of hydrilla and Eurasian watermilfoil, floating leaves of American pondweed, and 4-cm leaf segments of vallisneria. Total chlorophyll (a and b) was measured using a DMSO extraction procedure (Hiscox and Israelstam 1979).

Water samples were collected from all fluridone-treated tanks at 1, 2, 3, and 7 DAT, weekly thereafter through 42 DAT, and at 63 and 84 DAT to confirm initial fluridone treatment rates and to determine herbicide dissipation. Samples were collected in 500-ml amber polyethylene bottles and frozen until analysis. Fluridone residues were detected using a high performance liquid chromatography (HPLC) procedure<sup>2</sup>. Residue data were subjected to linear regression procedures and the results obtained were used to determine the half life of fluridone under these experimental conditions.

Treatments were randomly assigned to mesocosm tanks and were replicated three times. At each sampling interval, biomass and chlorophyll data were subjected to analysis of variance and treatment means compared using Fisher's protected LSD test at the 0.05 level of significance.

## RESULTS AND DISCUSSION

Residue analyses at 1 DAT (data not shown) showed that the initial target fluridone concentration (5 µg/L) was achieved in all chemically-treated mesocosm tanks. Subsequent water residue data were used to determine fluridone dissipation over time. Regression analysis established that under these experimental conditions, the average half life of fluridone in herbicide-treated tanks was 49 days ( $r^2 = 0.93$ ). Fluridone dissipation was comparable to dissipation rates reported by Netherland et al. (1997) under similar experimental conditions.

Treatment effects on dry weight biomass varied greatly among plant species (Figure 1A-D). The greatest response was observed on the target plant, hydrilla (Figure 1A). At 21 DAT, treatment with either fluridone alone or 200 CFU/ml *Mt* was sufficient to reduce hydrilla biomass by an average of 36%. However, the combined application of *Mt* plus fluridone reduced biomass up to 75% compared with untreated plants. By 84 DAT, the combined treatments resulted in a 93% reduction in hydrilla biomass. Both fluridone alone and 200 CFU/ml *Mt* reduced hydrilla biomass by 40% at the final harvest. Statistically, there were no differences between the two rates of *Mt* or between fluridone alone and *Mt* at 200 CFU/ml on hydrilla.

Characteristic injury symptoms of fluridone and *Mt* were observed on hydrilla. Successful fungal infection was noted on all *Mt*-treated tanks 10 DAT and was identified by leaf tip chlorosis and stem defoliation. Although biomass was not significantly different between the two rates of *Mt*, disease symptoms were visibly more abundant on tanks treated with the higher than the lower rate of *Mt*. At the first posttreatment harvest, new and healthy hydrilla growth (lateral shoots from viable stems) also was present in all tanks treated with *Mt* by

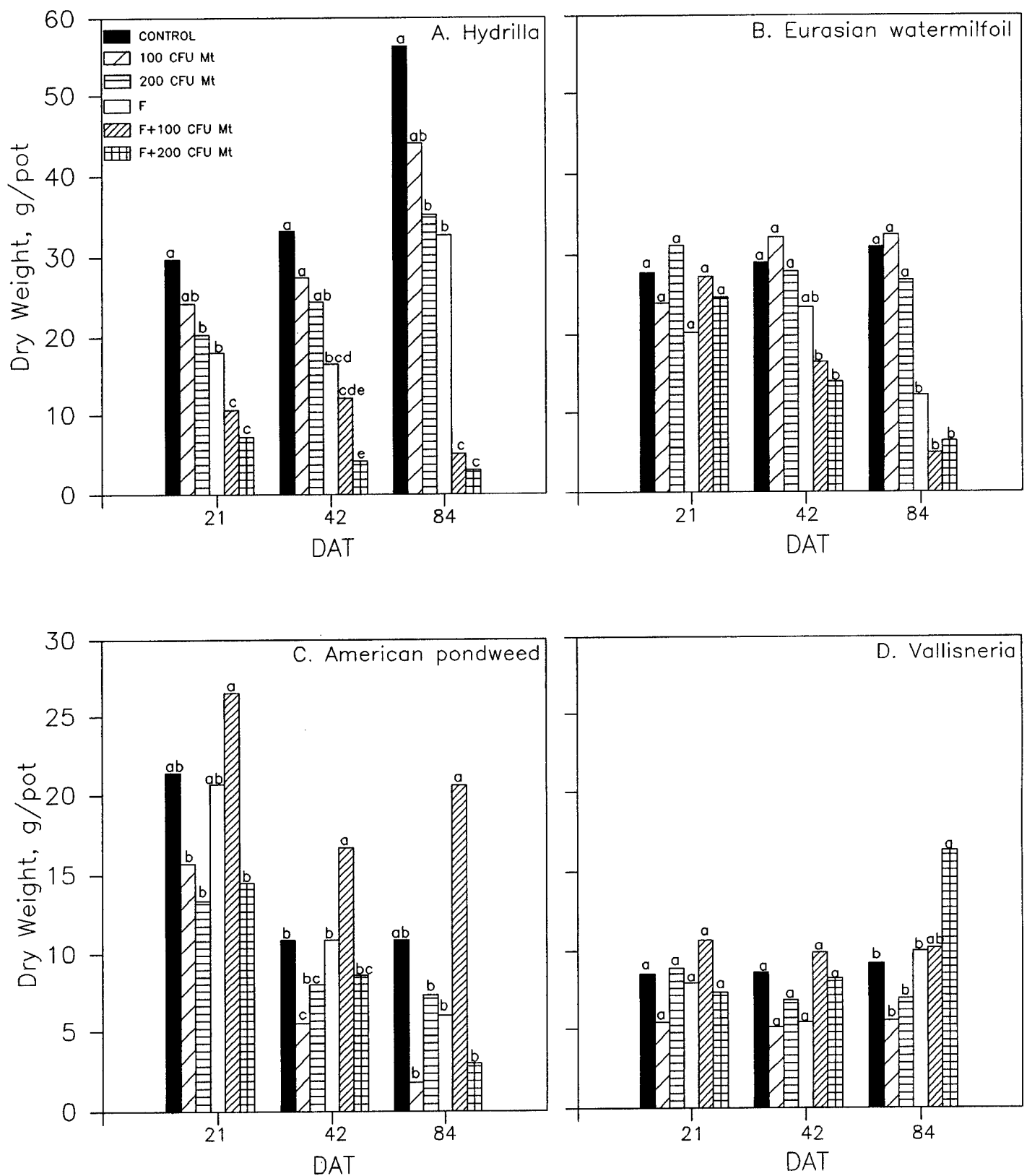


Figure 1. Mean dry weight biomass of hydrilla (A), Eurasian watermilfoil (B), American pondweed (C), and vallisneria (D) at 21, 42, and 84 days after treatment (DAT) following application of *Mt* at 100 and 200 colony forming units (CFU) per ml, fluridone (F = 5 µg/L fluridone), and integrated treatments of fluridone + *Mt*. Within each sample time, means followed by the same letter are not significantly different at  $P \leq 0.05$  according to Fisher's protected LSD test.

TABLE 1. EFFECT OF FLURIDONE, *Mt*, AND FLURIDONE + *Mt* TREATMENTS ON TOTAL CHLOROPHYLL CONTENT OF FOUR SUBMERSED PLANT SPECIES.

Species	Treatment $\mu\text{g/L} + \text{CFU}^1$	Total chlorophyll content ( $\text{mg g}^{-1} \text{ fr wt}$ )			
		Days after treatment <sup>2</sup>			
		Prettrt	21 DAT	42 DAT	84 DAT
Hydrilla	Untreated	1.17	1.11 a	1.09 a	1.12 a
	0 + 100	1.04	0.95 a	1.15 a	1.14 a
	0 + 200	1.02	0.97 a	1.03 a	1.22 a
	5 + 0	1.21	0.20 c	0.50 b	0.44 b
	5 + 100	1.14	0.30 bc	0.44 b	0.54 b
	5 + 200	1.16	0.39 b	0.52 b	0.56 b
	(LSD)	NS	(0.19)	(0.25)	(0.23)
E. Watermilfoil	Untreated	1.44	1.56 a	1.73 a	1.35 a
	0 + 100	1.58	1.53 a	1.70 a	1.51 a
	0 + 200	1.47	1.65 a	1.77 a	1.49 a
	5 + 0	1.40	1.05 b	1.03 b	0.98 b
	5 + 100	1.45	1.09 b	1.00 b	0.81 b
	5 + 200	1.50	1.06 b	1.14 b	0.92 b
	(LSD)	NS	(0.25)	(0.20)	(0.26)
American Pondweed	Untreated	1.42	1.10	1.40	1.43 b
	0 + 100	1.53	0.86	1.30	1.41 b
	0 + 200	1.74	0.97	1.40	1.51 b
	5 + 0	1.54	1.19	1.39	1.32 b
	5 + 100	1.70	1.11	1.30	1.27 b
	5 + 200	1.63	0.95	1.15	1.83 a
	(LSD)	NS	NS	NS	(0.32)
Vallisneria	Untreated	0.86	0.78 b	0.85 ab	0.68
	0 + 100	0.86	0.97 a	0.78 abc	1.35
	0 + 200	0.87	0.78 b	0.93 a	0.78
	5 + 0	0.87	0.52 c	0.66 bc	0.50
	5 + 100	0.92	0.62 c	0.64 bc	0.46
	5 + 200	0.74	0.51 c	0.58 c	0.63
	(LSD)	NS	(0.13)	(0.22)	NS

<sup>1</sup> $\mu\text{g/L}$  = fluridone concentration and CFU = colony forming units of *Mt*.<sup>2</sup>Within columns, means followed by different letters are significantly different (LSD,  $P \leq 0.05$ ); DAT, days after treatment; NS = not significant.

itself. Fluridone effects on hydrilla, pink stem coloration and bleached leaves on new tissues, were observed 21 DAT. Fluridone, but not *Mt*, symptomology was also observed on Eurasian watermilfoil. Neither vallisneria nor American pondweed displayed visible symptoms of fungal infection or fluridone leaf bleaching.

Although Eurasian watermilfoil was not the target plant in this study, treatment with fluridone alone and fluridone + either 100 or 200 CFU/ml *Mt* reduced Eurasian watermilfoil biomass by 75% at 84 DAT (Figure 1B). Unlike the synergistic effect observed on hydrilla, the response on Eurasian watermilfoil was likely due to fluridone itself as there were no statistical differences between treatments of fluridone alone and those integrated with *Mt*. The fact that effects on biomass were not observed until late in the study (42 DAT) further implies fluridone activity as the main source of efficacy. Fluridone is a slow acting herbicide in comparison to the quick infection response observed with *Mt* (Netherland and Shearer 1996). Results are consistent with other outdoor mesocosm studies in which fluridone at a rate of 5  $\mu\text{g/L}$  was sufficient to reduce Eurasian watermilfoil biomass (Netherland et al. 1997). Strains of *Mt* (other than that used in this study) have been isolated for activity on Eurasian watermil-

foil and were found to be effective in greenhouse trials (Gunner et al. 1990). Combining milfoil-specific strains of *Mt* with fluridone may have potential as an integrated approach for management of Eurasian watermilfoil, and should be evaluated.

Non-target species were less affected by fluridone and *Mt*. Compared with untreated plants, none of the treatments inhibited biomass of American pondweed at 21 DAT (Figure 1C). Results were variable at subsequent harvests. For example, *Mt* at 100 CFU/ml significantly reduced biomass by 50% 42 DAT while treatment with fluridone + 100 CFU/ml *Mt* resulted in a significant increase (35%) in biomass. By the end of the study, none of the treatments were statistically different than controls however, fluridone + 100 CFU/ml *Mt* showed significantly higher biomass when compared with other fluridone or *Mt* treatments. Some of the observed variation in biomass data can be attributed to insect damage. At 21 DAT, floating leaves of American pondweed had been severely decimated by an unidentified species of whitefly (*Trialeurodes* sp.) and a common aquatic insect identified as the larva of the waterlily leafcutter (*Synchlita oblitalis* (Walker)). Infestation was not evenly distributed among tanks (some tanks were not infested at all) and may account



for the variability in biomass data observed on this plant species. American pondweed in two of the three replicate tanks treated with fluridone + 100 CFU/ml *Mt* did not show insect damage, which may explain the high biomass levels recorded for this treatment.

*Vallisneria* biomass was not inhibited by any of the applied treatments (Figure 1D). There were no statistical differences among treatments at 21 and 42 DAT, and by the final harvest only fluridone + 200 CFU/ml *Mt* was significantly different than untreated plants. For reasons unknown, this treatment showed a 44% increase in biomass compared with untreated plants.

With the exception of American pondweed, all treatments that included fluridone significantly reduced total chlorophyll content in sampled tissues (Table 1). Hydrilla was most sensitive, with chlorophyll decreases of >70% measured at 21 DAT and a >50% decrease recorded thereafter. For Eurasian watermilfoil, chlorophyll content in fluridone-treated plants was 32 to 39% less than that of untreated plants throughout the study. Initially, *vallisneria* showed reduced leaf chlorophyll (by 29% at 21 DAT), however, at 84 DAT there were no differences among treatments, indicating plant recovery. For all plant species, *Mt* alone did not affect total chlorophyll at the times sampled. Netherland and Shearer (1996) showed reduced chlorophyll content in hydrilla at 7 and 14 days following treatment with 100 and 200 CFU/ml *Mt*, but the effects dissipated by 28 DAT.

The results of this study confirm those observed in growth chamber studies by Netherland and Shearer (1996). For hydrilla, a beneficial synergistic interaction was observed with combined applications of 5 µg/L fluridone with either 100 or 200 CFU/ml *Mt*. Neither control agent alone provided adequate hydrilla control. For Eurasian watermilfoil, 5 µg/L fluridone was sufficient to significantly reduce biomass, which was consistent with reports that maintenance of low doses of fluridone over time can significantly inhibit biomass production (Netherland et al. 1997). There was no advantage to integrating fluridone with *Mt* on Eurasian watermilfoil. At the rates applied, the strain of *Mt* utilized in this study was ineffective on Eurasian watermilfoil. Other strains of *Mt* have been isolated for pathogenicity on this plant species and may be potential candidates for integrating with fluridone.

The desired level of selectivity was achieved with the integrated treatments applied in this study. Biomass of American pondweed and *vallisneria* was not severely impacted by treatment rates sufficient to control the target species, hydrilla. The results demonstrated that by integrating fluridone and *Mt*, a low herbicide rate that reduced the likelihood of chemical damage to non-target species could be used. The poten-

tial for selectivity gives further merit to the concept of integrated weed management.

Future research will focus on larger-scale field testing of fluridone-*Mt* treatments for controlling hydrilla, as well as evaluating other potential herbicide-pathogen combinations for aquatic plant management. Development of a granular *Mt* formulation to provide an easier and more uniform means of application also has been initiated.

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# Computer Tools Developed for Aquatic Plant Management

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## ABSTRACT

Over the past fifteen years, the Aquatic Plant Control Research Program (APCRP) has pursued development of personal computer (PC) -based information systems for technology transfer in aquatic plant management. Initial development was limited to DOS compatible simulation models and other computational tools. Though effective in generating computational information for several control techniques, the DOS operating system did not allow the models to be interlinked with other information sources required for development of effective aquatic plant management technology transfer tools. However, the advent of the WINDOWS operating system and ensuing software advances for PC's have allowed the recent development of more comprehensive information systems. Of these, the Aquatic Plant Information System (APIS) is the first system scheduled for release for the APCRP. In addition to the simulation models developed in the past (i.e., HARVEST, AMUR/STOCK, and HERBICIDE), the system will include both aquatic plant and biological control identification strategies based on expert system programming, instructional information on a diversity of aquatic plant management topics, and other utilities all accessible through an online HELP supported graphical user interface. The system will be distributed on CD-ROM. Additional DOS-based models not converted to a WINDOWS-compatible format (INSECT, HYDRIL, and MILFO) will be also be included for direct installation from the CD-ROM.

**Key words:** aquatic plant management, information systems, technology transfer tools, simulation models, expert systems.

## INTRODUCTION

Over the past fifteen years, the Waterways Experiment Station (WES), in support of the Aquatic Plant Control Research Program (APCRP), has developed a series of personal computer (PC) -based tools to facilitate aquatic plant management technology transfer. The goal of these tools is to provide government agencies and private firms and associations involved with aquatic plant management information that will help them plan and implement environmentally

sound management programs that are both effective and economically efficient.

Development of these PC-based tools has proceeded along the lines of technological advances in PC architecture and operating systems. Initially, tools were limited to simulation models and related procedures which were heavily computational. Though numeric outputs were useful information, their applicability was restricted both spatially and temporally to a defined point and time. Extrapolation of model outputs to other locations, or to the same location at a different time, was tenuous. Further, processing of other types of information was extremely limited. As instructional tools, PC's were actually less effective for packaging textual and graphical/visual data than text books, reports, or other forms of printed media. However, recent improvements in information packaging and management made available by current multimedia PC platforms have expanded the potential opportunities for developing PC-based information systems. This manuscript presents a chronological sequence in PC-based technology transfer tool development by WES for the APCRP.

## SIMULATION MODEL DEVELOPMENT

The Waterways Experiment Station (WES) investigated development of computer-based simulation models for evaluating growth of nuisance aquatic plants through a workshop held in 1980 (Wlosinski 1981). As a result, a generic submersed plant growth model was developed (Collins et al. 1985). The first model developed by WES of an operational technique for controlling nuisance growth of aquatic plants was the HARVEST model (Hutto 1982, Sabol 1983). This model includes algorithms that simulate aquatic plant control cutting operations, collecting operations, and transport operations (i.e., both over-water and road-based). As an analytical tool, the HARVEST model allows users to test overall operational productivity (i.e., acres or tons of plant material harvested per hour) of existing mechanical harvesting systems under "user-defined" operational and environmental conditions. Initially developed for a mainframe computer, HARVEST was modified in 1982 for installation on DOS-based personal computers, and was cleared for public distribution in 1983. HARVEST has been converted to a WINDOWS-based format.

In addition to the HARVEST model, WES also began distributing a grass carp stocking rate model (STOCK) in 1983. Development of this model at WES coincided with the large-scale operations management test (LSOMT) of grass carp stockings for hydrilla control in Lake Conway, Florida. The STOCK model (Miller and Decell 1984) was intended to be

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used as a tool whereby an aquatic plant resource manager could "define" selected growth characteristics of a targeted hydrilla infestation (e.g., area of infestation and seasonal "biomass" growth pattern) and evaluate the effectiveness of different grass carp stocking strategies in controlling that infestation. The AMUR/STOCK model has been expanded to allow stocking strategy evaluations for controlling other target plant species (Stewart and Boyd 1994), and was recently converted to operate under the WINDOWS operating system.

Based on the ability of the HARVEST and STOCK models to help aquatic resource managers evaluate application strategies for the above two operational control techniques, development of additional simulation capabilities were planned for other "operationally proven" techniques. HERBICIDE, a simulation tool for evaluating herbicide application techniques, generates estimates of the post-application partitioning (water, sediments, and plants) and dissipation of the active ingredient fraction of an herbicide formulation following application. Examples of intended applications of this model are provided in Stewart (1994). Developed initially as a DOS-based model, HERBICIDE has been converted to a WINDOWS-based format (Stewart and McAllister 1995).

Development of a simulation model for two insect species introduced in the United States for the biological control of waterhyacinth was also undertaken at WES. The INSECT model (Akabay et al. 1988) provides a multiple-year simulation of the population dynamics of waterhyacinth and two introduced weevil species. In comparison to the STOCK model, the INSECT model represents a significantly more complex biological system (e.g., reproductive viability and multiple generations and life stages of the control agents). Though the model has been released in DOS-based format (Stewart and Boyd 1992), knowledge gaps in our understanding of many of the governing mechanisms of this system have necessitated the inclusion of several "black box" relationships and a rigid set of assumptions under which the model is valid. For these reasons, INSECT has had limited success as a technology transfer tool. Its main utility to date has been to identify knowledge gaps in our understanding of the population dynamics of the two weevil species and their mode of impacting waterhyacinth growth (Howell and Stewart 1989; Grodowitz and Stewart 1989).

Due to its modular construction, the INSECT model also provides an independently functional, DOS-based plant growth model for waterhyacinth. DOS-based plant growth models have also been developed for hydrilla (HYDRIL; Best and Boyd 1996) and for Eurasian watermilfoil (MILFO; Best and Boyd 1997). As independently functioning models, these simulation tools were designed to help users understand how selected environmental site conditions affect the growth of these nuisance plant species. Through a better understanding of these relationships achieved through hands-on model evaluations, it was hoped that users could design better control strategies for managing aquatic vegetation.

### **SIMULATION MODEL LIMITATIONS**

Though successful in many respects, simulation model development as the main means of PC-based technology

transfer had several limitations. First, development of the models was painstakingly slow because this depended on the availability of a relatively comprehensive knowledge of the systems being modeled. Because knowledge was typically lacking for some of the key processes, a given model's applicability was restricted by a set of assumptions which defined the limited conditions under which the model was valid. Further, many of the processes represented by the simulations (e.g., plant photosynthesis, water exchange, etc.) are driven by environmental and biotic conditions that are both spatially and temporally heterogeneous. For this second reason, coupled with the fact that spatial databases (e.g., geographic information systems and hydrodynamic databases) had memory requirements in excess of what could be provided on PC's, the utility of the models was often limited to "generalized" initialization conditions.

Due to these and other limitations, packaging of the models as PC-based technology transfer tools was initially hampered by limitations in PC technology. What was needed was a PC platform that not only would allow execution of the models, but one that could also include support information for operating the models and interpreting their outputs. In general, prior to the introduction of the WINDOWS operating system and object oriented programming tools, IBM compatible PC systems were not capable of performing these other functions. These same limitations in pre-WINDOWS PC systems also prevented them from being a practical platform for packaging other types of information (e.g., control technique "how to guides", plant identification information, aquatic plant and biocontrol agent life history and ecological data) that should be included in comprehensive aquatic plant management technology transfer tools.

### **NEW APPROACHES FOR INFORMATION SYSTEMS**

New capabilities provided by the WINDOWS operating system and related object oriented software tools allowed for the development of new information systems that had not been possible on DOS-based systems. The first true information package pursued by APCRP for aquatic plant management was the Aquatic Plant Resource and Operations On-line System (APROPOS, Madsen and McAllister 1995). APROPOS was designed to provide a consistent procedure for evaluating pertinent information for development of aquatic plant management plans. The different types of information to be included in the system were to be arranged in separate, but interlinked modules accessible through a graphical user interface (GUI) shell operating under WINDOWS 3.1. Through point and click selection, the system would provide access to a management strategy planning module which helped identify the particular problem species and then access other "toolboxes" in the system for further information. Other toolboxes for the planned system were to provide access to a literature database on plant life history and ecological data, a literature database specific to control technique options, and a literature database which presented "how to" guides on developing sampling plans and carrying out field data collection efforts. A simulation model toolbox, a spatial database toolbox, and a HELP facility toolbox for instructions on system operation had also been planned. In planning APROPOS, it was envisioned that

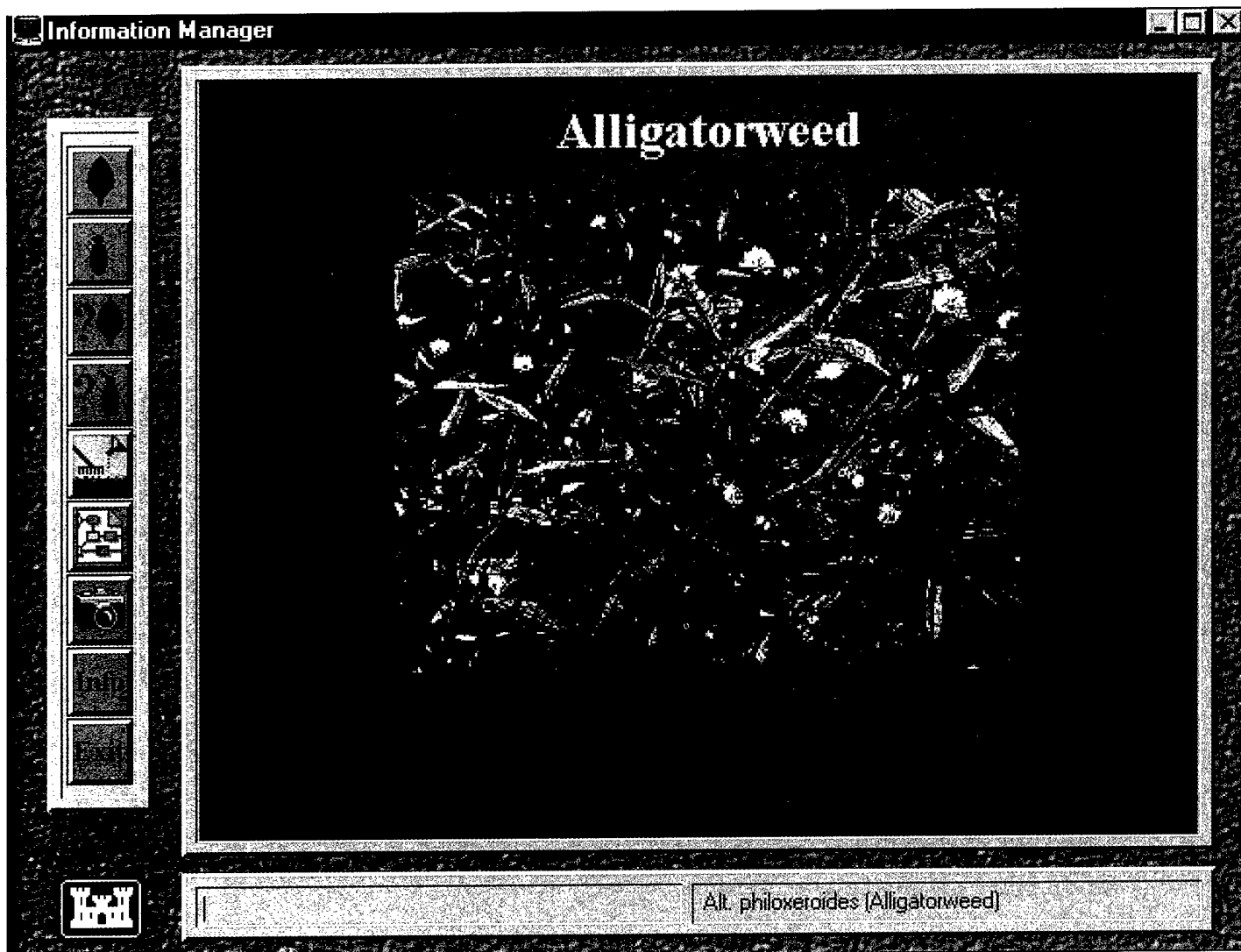


Figure 1. Information manager screen of the Aquatic Plant Information System (APIS). Access to information in APIS is achieved through mouse activation of the appropriate icon on the left side of the screen.

all models previously developed by WES for the APCRP would be converted for WINDOWS operation and would be accessible through the simulation model toolbox. However, development of APROPOS would have taken considerable time and resources. Due to the reduction in APCRP funding beginning in 1995, this extensive and long-term investment did not appear reasonable.

Concurrent to our planning and initial development of APROPOS, other PC based information systems were being developed at WES to facilitate technology transfer. Both the Noxious and Nuisance Plant Management Information System (PMIS) (Grodowitz et al. 1996) and the Zebra Mussel Information System (ZMIS) (Grodowitz et al. 1997) contain a variety of information on the management of troublesome pest species. For various reasons, development of these systems proceeded more rapidly than development of APROPOS. Operational CD-ROM versions of both PMIS and ZMIS have been widely distributed, and initial reviews of both systems have been highly favorable.

Resources scheduled for further development of APROPOS were redirected toward development of an aquatic plant management information system similar in design to ZMIS and PMIS. The newly designed system, which incorporated existing information, programs, and expertise, as well as the demonstration material for APROPOS, has been titled the Aquatic Plant Information System (APIS).

### DESCRIPTION OF APIS

APIS will operate under Windows 3.1 or Windows 95. The system, which was developed using a combination of Borland's® C++ and Microsoft's® Visual Basic, will operate using a 386 processor but a 486SX 25 MHz (or faster) utilizing 8 MB of RAM is recommended. The system will be contained on a single CD-ROM and can be loaded entirely on your hard drive using a 2X (or faster) drive. APIS will also run directly from the CD-ROM thereby limiting hard drive space requirements from about 100 MB (i.e., a full install) to a minimum

TABLE 1. SIMULATION MODELS RELEASED WITH THE AQUATIC PLANT INFORMATION SYSTEM (APIS).

Model	Description	Status	Integrated or Stand-Alone	Reference
Harvest	Mechanical harvesting model	Both DOS and WINDOWS version available	Integrated	Hutto 1982, Sabol 1983
Amur/Stock	Grass carp stocking model	DOS and WINDOWS versions available	Integrated	Miller and Decell 1994, Stewart and Boyd 1994
Herbicide	Herbicide dissipation model	WINDOWS version available	Integrated	Stewart 1994, Stewart and McAllister 1995
Insect	Insect biocontrol model for waterhyacinth	DOS/ FORTRAN version available	Stand-alone	Akbay et al. 1988, Stewart and Boyd 1992
Hydril	Hydrilla plant growth model	DOS/ FORTRAN version available	Stand-alone	Best and Boyd 1996, Boyd and Best 1996
Milfo	Eurasian watermilfoil plant growth model	DOS/ FORTRAN version available	Stand-alone	Best and Boyd 1997

of only 1 MB. Since large numbers of color images are included in APIS, your system must contain a video board, video drivers, and monitor capable of displaying a minimum of 256 colors simultaneously for Windows 3.1 and 65,000 colors simultaneously for Windows 95. Information/image access will be relatively rapid even when using APIS with minimum system requirements and operating it directly from the CD-ROM. Since the system will be highly graphical in nature a mouse or similar pointing device also will be required. Installation instructions will be straight forward and contained on the inner title page of the CD-ROM cover. While APIS will be highly intuitive to operate, detailed instructions for system operation will be contained in hyper-linked text files which can be accessed directly from the program.

Accessing information in APIS is accomplished through mouse selection of icon choices included on the left hand side of the "Information Manager" screen depicted in Figure 1. Through these icons, the user gains access to system tools which perform the following respective functions. The top icon allows the user to select a plant species of interest from the list of over sixty species included in the system. Information can then be obtained on the plant's distribution, history of introduction, and textual description, as well as full color images. If the user is unsure of the plant species in question, the third icon provides a link to an identification tool. If biological control agents are available for the plant species, information regarding their identification is obtained through the second and fourth icons. Once the plant species is determined, the fifth icon provides access to helpful information on biological, chemical and mechanical control options. The sixth icon provides access to WINDOWS compatible versions of several of the simulation models described earlier in this manuscript. As an aid to species identifications, the seventh icon accesses high quality images of each of the aquatic plant species and their included bio-control agents. Incorporation of the models within APIS facilitates many of the user-friendly execution features as envisioned through APROPOS. Simulation models accessible through APIS are identified in Table 1.

#### AVAILABILITY OF APIS

A limited test release of APIS during December 1997 will be followed by a full distribution of the system during the latter half of 1998. The system will be distributed on CD-ROM, and should function on most personal computers utilizing the WINDOWS 3.1 or WINDOWS 95 operating systems.

Those interested in receiving a copy of APIS can submit a request (by standard mail, telephone, FAX, or E-mail) with name and address to: Dr. Michael J. Grodowitz, U.S. Army Engineer Waterways Experiment Station, ATTN: CEWES-ER-A, 3909 Halls Ferry Road, Vicksburg, MS 39180-6199, phone (601) 634-2972, FAX (601) 634-2398, or E-mail at grodowm@mail.wes.army.mil.

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# Response of Littoral Fishes in Upper Lake Marion, South Carolina Following Hydrilla Control by Triploid Grass Carp

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## ABSTRACT

A seven-year study in upper Lake Marion, South Carolina evaluated the response of fishes to hydrilla (*Hydrilla verticillata* (L. f.) Royle) removal by triploid grass carp (*Ctenopharyngodon idella* Valenciennes). A boat-mounted electroshocker was used to quantify relative abundance and species composition of fishes at 10 permanent locations distributed throughout the upper lake. A total of 16,306 fish representing 64 species were collected. The taxonomically dominant family was Centrarchidae and the numerically dominant family was Clupeidae. There were significant differences in catch between years with high and low hydrilla coverage. Littoral fishes, especially Centrarchidae, increased as hydrilla decreased from a maximum of 4,700 ha (approximately 50% of the surface area) to less than 100 ha by 1994. Mean lengths of most littoral species were similar during the study. Despite substantial declines in hydrilla, other forms of cover

persisted during the study providing an intermediate level of structural complexity. Consequently, grass carp effectively controlled hydrilla but did not create any detectable negative effects on the littoral fish assemblage during the study.

**Key words:** plant coverage, electroshocking, largemouth bass, multi-year.

## INTRODUCTION

Hydrilla became established in upper Lake Marion during the early 1980's (de Koslowski 1994) and by 1988 had colonized over 4,000 ha. In 1989, triploid grass carp were stocked into upper Lake Marion to control hydrilla. By 1994, almost 600,000 fish had been released into the Santee Cooper system (Lakes Marion, Moultrie, and the connecting canal). With an annual mortality of approximately 20%, the 1994 density of triploid grass carp in the Santee Cooper system was estimated at 17 fish per vegetated ha (Morrow et al. 1997). Extensive surface coverage of hydrilla persisted through 1991, began to decline in 1992, and was reduced to less than 60 ha in upper Lake Marion by 1994 (S. de Koslowski, South Carolina Department of Natural Resources, personal communication).

Triploid grass carp have also been found to be an effective biocontrol technique in other water bodies (Allen and Wat-

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tendorf 1987, Wattendorf and Anderson 1987). However, achieving appropriate densities of grass carp can be difficult, and stocking results can vary from no control to total elimination of vegetation (Sutton 1977, Leslie et al. 1987, Santha et al. 1991, Kirk 1992). Adverse impacts on fish communities, particularly sport fishes, are commonly cited as an environmental concern (Fedorenko and Fraser 1978, Gasaway 1978, Ware and Gasaway 1978, Bain 1993). Angling is an important recreational activity in the Santee Cooper system (Sample 1990) as well as in most vegetated water bodies. Largemouth bass (*Micropterus salmoides* Lacepède) anglers prefer to fish around aquatic vegetation, particularly in areas devoid of any other cover (Wilke et al. 1992, Maccina and Reeves 1996). Consequently, any control activities, particularly using grass carp, are controversial because of potential negative effects on angling.

We sampled fish in upper Lake Marion for seven years to evaluate effects of decreasing hydrilla coverage on fish abundance. Unlike unvegetated water bodies colonized by exotic plants, upper Lake Marion had substantial amounts of standing timber and native aquatic plants prior to the establishment of hydrilla. Electroshocking was used to determine relative abundance of littoral fishes from 1988 when hydrilla dominated most of the littoral zone, to 1994 when hydrilla was virtually eliminated. Relative abundance of fish species, including largemouth bass, were compared among years to determine long-term response of the fish community to large-scale changes in vegetation coverage.

## MATERIALS AND METHODS

The 70,000 ha Santee Cooper system was created in 1941 for hydropower and flood control. The study site, upper Lake Marion, is approximately 10,000 ha located from the confluence of the Wateree and Congaree Rivers downstream to the Interstate 95 bridge (Figure 1). This portion of the lake is mostly shallow (<2 m deep) with substantial amounts of standing timber and has historically supported abundant native aquatic vegetation. During the study, the dominant submersed aquatic vegetation was hydrilla but other aquatic species included Brazilian elodea (*Egeria densa* Planch.), coontail (*Ceratophyllum demersum* L.), slender naiad (*Najas minor* All.), pondweed (*Potamogeton* spp.), and water primrose (*Ludwigia* spp.). Coverage of submersed vegetation visible from the surface was estimated yearly (except for 1989) from either aerial photography or maps prepared from overflights. Ground truth surveys were conducted to verify maps. The estimated coverage for 1988, 1990, 1991, 1992, 1993, and 1994 was 4,170, 4,800, 4,700, 1,580, 323, and 60 ha, respectively (S. de Kozlowski, South Carolina Department of Natural Resources, personal communication). Coverage for 1989 was interpolated using 1988 and 1990 estimates.

Permanent sites were distributed throughout upper Lake Marion to represent the different levels of hydrilla coverage (Figure 1). Sites 1 and 2 were located at the extreme upper end of the lake, called Sparkleberry Swamp; surface coverage of hydrilla was moderate. Site 3 was located on the main stem of the Santee River near a railroad trestle and had the least amount of plants. The remaining sites had extensive hydrilla coverage prior to elimination by grass carp. Site 4 was situated between Pack's and Elliott's flats; Site 5 was located at Elliott/Bee cut; and site 6 was located in Stump Hole Swamp

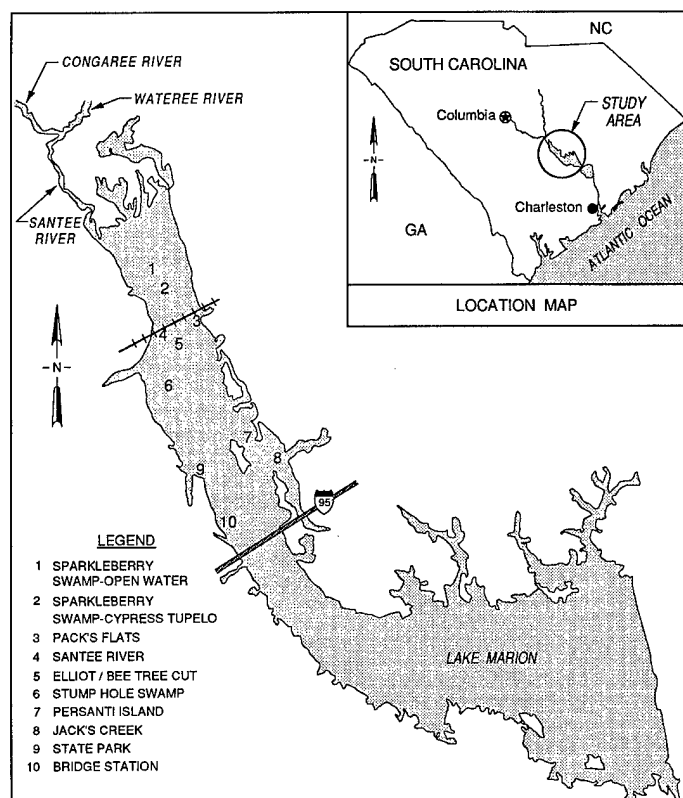


Figure 1. Location of electroshocking sampling stations in upper Lake Marion, South Carolina.

where herbicides had been used to control hydrilla. Sites 7 and 8, Persanti Island and Jack's Creek, were added in 1989 and sites 9 and 10 found near the State Park and Interstate 95 bridge, were added in 1990. Sampling locations within each site remained the same throughout the study.

A boat-mounted electroshocker using pulsed, direct current of approximately 400 v at 3 to 6 amps was used to collect fish. Each site was sampled for 15 min on each sampling date and two dippers attempted to retrieve all fish. Except in 1988, each site was repeatedly sampled during the plant growing season (April through November) resulting in the following sample size: 1988 = 5, 1989 = 16, 1990 = 45, 1991 = 40, 1992 = 10, 1993 = 20, 1994 = 40. Electroshocking usually occurred in boat lanes adjacent to plant beds or in sub-surface vegetated zones; dense mats of vegetation were avoided because of a lower dipping efficiency in these habitats. All stunned fish were identified, counted, measured for total length, and released.

Because electroshocking is a sampling technique for shallow waters, littoral species are more vulnerable than pelagic species (Reynolds 1983). Thus, catch-per-unit-effort (CPUE, 15 min shocking) was statistically analyzed for the following taxa: all species combined, all littoral species combined, and individual littoral species commonly collected during the study (>0.8% of total number collected); individual pelagic species were excluded. Catch-per-unit-effort was log transformed to adjust for heterogeneity of variances. After log transformation, normality was verified using the Shapiro-Wilk statistic (SAS 1985).



Catch-per-unit-effort was compared between high (>4,000 ha, 1989-1990) and low (<500 ha, 1993-1994) hydrilla coverage using a repeated-measures analysis of variance. This procedure was selected because observations at fixed stations within each time period were not independent (Maceina et al. 1994). In addition to similar sample sizes, time periods represented pretreatment and posttreatment conditions relative to vegetation control by grass carp. The transition years of 1991-1992 were excluded to increase the likelihood that 1989-1990 and 1993-1994 were independent samples. Mean lengths of the 10 most common species were similarly tested. Statistical significance was set at  $P < 0.1$  because of high variability associated with electroshocking data and the patchy distribution of fishes in aquatic plants.

## RESULTS

We collected 16,306 fish representing 64 species in 176, 15-min electroshocking samples (Table 1). The taxonomically dominant family was Centrarchidae, comprising 15 species and accounting for 22% of the total number of fish collected. The numerically dominant family was Clupeidae, comprising five species and accounting for 37% of the total number of fish collected. Other common families included Cyprinidae and Catostomidae. Dominant species ( $\geq 4\%$ ), in decreasing order of abundance, were: threadfin shad (*Dorosoma petenense* Günther), golden shiner (*Notemigonus crysoleucas* Mitchell), gizzard shad (*Dorosoma cepedianum* Lesuer), largemouth bass, bluegill (*Lepomis macrochirus* Rafinesque), eastern silvery minnow (*Hybognathus regius* Girard), blueback herring (*Alosa aestivalis* Mitchell), redear sunfish (*Lepomis microlophus* Günther), and inland silverside (*Menidia beryllina* Cope).

Number of species collected during high and low hydrilla coverage was similar (51 and 50 species, respectively). However, mean catch of all species combined significantly increased during low hydrilla coverage (Table 2). Littoral fishes showed similar results. Frequently collected littoral species (>0.8% of total catch) that increased significantly after hydrilla declined included bowfin (*Amia calva* Linnaeus), golden shiner, lake chubsucker (*Erimyzon sucetta* Lacépède), bluegill, redear sunfish, largemouth bass, and yellow perch (*Perca flavescens* Mitchell). Mean catch of coastal shiner (*Notropis petersoni* Fowler) and black-spotted sunfish (*Lepomis punctatus* Valenciennes) also increased significantly during low hydrilla coverage, but to a lesser degree. There was no significant difference in mean catch of chain pickerel (*Esox niger* Lesueur) between the two time periods.

Of the ten frequently collected littoral species, total length of coastal and golden shiners were significantly higher during low hydrilla coverage (Figure 2). However, mean total length ( $\pm$  SD) of largemouth bass was significantly higher during high hydrilla coverage (207 mm  $\pm$  130), compared to low coverage (180 mm  $\pm$  137). There were no significant differences in total length for the remaining seven littoral species.

## DISCUSSION

Effects of large-scale control of submersed aquatic plants on fishes are limited to a few conflicting studies (Dibble et al. 1996). Bailey (1978) suggested that variables such as weather,

TABLE 1. FISHES COLLECTED AT UPPER LAKE MARION, SOUTH CAROLINA FROM 1988-1994 USING A BOAT-MOUNTED ELECTROSHOCKER. TOTAL NUMBER COLLECTED AND HABITAT GUILD ARE INDICATED (L = LITTORAL, L/P = LITTORAL/PELAGIC, P = PELAGIC). ONLY LITTORAL (L AND L/P) SPECIES REPRESENTING MORE THAN 0.8% OF TOTAL NUMBER COLLECTED (>16 INDIVIDUALS), EXCLUDING GRASS CARP, WERE INCLUDED IN STATISTICAL ANALYSIS.

Family and Species	Total Number Collected	Habitat Guild
Lepisosteidae		
Longnose gar ( <i>Lepisosteus osseus</i> Linnaeus)	173	P
Amiidae		
Bowfin ( <i>Amia calva</i> Linnaeus)	235	L
Anguillidae		
American eel ( <i>Anguilla rostrata</i> Lesueur)	3	L/P
Clupeidae		
Blueback herring ( <i>Alosa aestivalis</i> Mitchell)	771	P
Hickory shad ( <i>A. mediocris</i> Mitchell)	3	P
American shad ( <i>A. sapidissima</i> Wilson)	260	P
Gizzard shad ( <i>Dorosoma cepedianum</i> Lesuer)	1718	P
Threadfin shad ( <i>D. petenense</i> Günther)	3333	P
Cyprinidae		
Grass carp ( <i>Ctenopharyngodon idella</i> Valenciennes)	25	L/P
Whitefin shiner ( <i>Cyprinella nivea</i> Cope)	18	L
Common carp ( <i>Cyprinus carpio</i> Linnaeus)	51	P
Eastern silvery minnow ( <i>Hybognathus regius</i> Girard)	964	P
Golden shiner ( <i>Notemigonus crysoleucas</i> Mitchell)	1828	L/P
Ironcolor shiner ( <i>Notropis chalybaeus</i> Cope)	3	L
Spottail shiner ( <i>N. hudsonius</i> Clinton)	9	L
Taillight shiner ( <i>N. maculatus</i> Hay)	73	L/P
Coastal shiner ( <i>N. petersoni</i> Fowler)	367	L
Fathead minnow ( <i>Pimephales promelas</i> Rafinesque)	1	L
Creek chub ( <i>Semotilus atromaculatus</i> Mitchell)	3	L/P
Catostomidae		
White sucker ( <i>Catostomus commersoni</i> Lacépède)	2	P
Creek chubsucker ( <i>Erimyzon oblongus</i> Mitchell)	3	L
Lake chubsucker ( <i>E. sucetta</i> Lacépède)	412	L
Highfin carpsucker ( <i>Carpiodes velifer</i> Rafinesque)	2	P
Smallmouth buffalo ( <i>Ictiobus bubalus</i> Rafinesque)	1	P
Spotted sucker ( <i>Minytremma melanops</i> Rafinesque)	276	P
Ictaluridae		
Yellow bullhead ( <i>Ameiurus melas</i> Lesueur)	1	L/P
Brown bullhead ( <i>A. nebulosus</i> Lesueur)	10	L
Blue catfish ( <i>Ictalurus furcatus</i> Lesueur)	17	P
Channel catfish ( <i>I. punctatus</i> Rafinesque)	12	P
Fleathad catfish ( <i>Pylodictis olivaris</i> Rafinesque)	5	L/P
Esocidae		
Redfin pickerel ( <i>Esox americanus americanus</i> Gmelin)	5	L
Chain pickerel ( <i>E. niger</i> Lesueur)	150	L
Umbridae		
Eastern mudminnow ( <i>Umbra pygmaea</i> DeKay)	3	L
Aphredoderidae		
Pirate perch ( <i>Aphredoderus sayanus</i> Gilliams)	15	L
Belontiidae		
Atlantic needlefish ( <i>Strongylura marina</i> Walbaum)	2	P
Cyprinodontidae		
Golden topminnow ( <i>Fundulus chrysotus</i> Günther)	60	L
Lined topminnow ( <i>F. lineolatus</i> Agassiz)	1	L
Poeciliidae		
Eastern mosquitofish ( <i>Gambusia holbrooki</i> Girard)	296	L
Least killifish ( <i>Heterandria formosa</i> Agassiz)	7	L
Atherinidae		
Brook silverside ( <i>Labidesthes sicculus</i> Cope)	129	P
Inland silverside ( <i>Menidia beryllina</i> Cope)	606	P
Percichthyidae		
White perch ( <i>Morone americana</i> Gmelin)	399	P
White bass ( <i>M. chrysops</i> Rafinesque)	10	P
Striped bass ( <i>M. saxatilis</i> Walbaum)	30	P
Centrarchidae		
Flier ( <i>Centrarchus macropterus</i> Lacépède)	9	L
Blackbanded sunfish ( <i>Enneacanthus chaetodon</i> Baird)	50	L



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Family and Species	Total Number Collected	Habitat Guild
Bluespotted sunfish ( <i>E. gloriosus</i> Holbrook)	44	L
Banded sunfish ( <i>E. obesus</i> Girard)	7	L
Redbreast sunfish ( <i>Lepomis auritus</i> Linnaeus)	10	L
Green sunfish ( <i>L. cyanellus</i> Rafinesque)	2	L
Pumpkinseed ( <i>L. gibbosus</i> Linnaeus)	20	L
Warmouth ( <i>L. gulosus</i> Cuvier)	88	L
Bluegill ( <i>L. macrochirus</i> Rafinesque)	1167	L
Dollar sunfish ( <i>L. marginatus</i> Holbrook)	17	L
Redear sunfish ( <i>L. microlophus</i> Günther)	783	L
Black-spotted sunfish ( <i>L. punctatus</i> Valenciennes)	154	L
Largemouth bass ( <i>M. salmoides</i> Lacepède)	1224	L
White crappie ( <i>Pomoxis annularis</i> Rafinesque)	3	L/P
Black crappie ( <i>P. nigromaculatus</i> Lesueur)	46	L/P
Percidae		
Tessellated darter ( <i>Etheostoma olmstedii</i> Storer)	7	L
Swamp darter ( <i>E. fusiforme</i> Girard)	3	L
Sawcheek darter ( <i>E. serrifer</i> (Hubbs and Cannon)	2	L
Yellow perch ( <i>Perca flavescens</i> Mitchill)	346	L
Mugilidae		
Striped mullet ( <i>Mugil cephalus</i> Linnaeus)	32	P
Total:	16306	

water level fluctuation, trophic status, and fishing pressure contribute to uncertainty in predicting the response of fishes to declining vegetation. Water body size can also influence fish-plant interactions. The importance of aquatic macrophytes to the overall functioning of lakes decreases proportionately as lakes get larger and deeper (Hoyer and Canfield 1996) suggesting that fish-plant interactions are more difficult to predict in larger water bodies and comparative studies are likely to result in different patterns of species abundance.

Grass carp stocked into Lake Conroe, TX, which encompasses 8,100 ha, completely eliminated hydrilla resulting in a numerical increase of pelagic species and a decline in some littoral species (Noble 1986, Bettoli et al. 1993). Bailey (1978) and Shireman et al. (1985) detected no changes or conflicting patterns of species abundance as vegetation declined in larger impoundments in Arkansas and Florida, respectively. Unlike most of these lakes, which lacked substantial structure prior to infestation of exotic plants, moderate amounts of submersed structure remained in upper lake Marion and thus, we suggest, contributed to an increase in abundance of littoral fishes.

Moderate densities of vegetation, which reportedly range from 10 to 40% coverage of the surface area, provide spatial complexity that promote fish diversity and is optimal for sport fish production (Hestand and Carter 1978, Crowder and Cooper 1979a, 1979b, Wiley et al. 1984). Most sunfishes, including largemouth bass, bluegill, redear sunfish, and black-spotted sunfish, are structurally-oriented so their abundance is often positively correlated with vegetation coverage (Ware and Gasaway 1978, Borawa et al. 1979, Forester and Lawrence 1978, Noble 1986, Durocher et al. 1984, Wiley et al. 1984, Moxley and Langford 1985, Klussman et al. 1988, Scott 1993). Consequently, phytophilic sunfishes decrease in abundance after removal of vegetation (Bettoli et al. 1993, Ware and Gassaway 1978) but this response was not observed in our study. Although grass carp reduced surface coverage of hydrilla in upper Lake Marion from approximately 50% to less than 10%, abundant structure in the form of standing timber, sub-surface submersed vegetation, and floating and emergent species near the shoreline remained (S. de Kozlowski, South Carolina Department of Natural Resources, personal communication). Thus, the underwater landscape of upper Lake Marion was shifted from monospecific stands of hydrilla to intermediate levels of structural complexity.

Dense hydrilla contributes to stunted populations (Colle and Shireman 1980), but intermediate levels of structural complexity allow juvenile sunfish to forage in vegetated regions on soft-bodied organisms (Mittelbach 1981) and

TABLE 2. MEAN NUMBER ( $\pm$  SD) OF FISH PER 15 MINUTES OF ELECTROSHOCKING COLLECTED IN UPPER LAKE MARION, SOUTH CAROLINA, 1988-1994. PROBABILITIES (P) INDICATE SIGNIFICANCE BETWEEN HIGH (1989-1990) AND LOW (1993-1994) HYDRILLA COVERAGE USING REPEATED-MEASURES ANALYSIS OF VARIANCE. VEGETATION COVERAGE IS THE AERIAL AMOUNT OF SUBMERSED AQUATIC VEGETATION DETERMINED DURING SEPTEMBER OR OCTOBER OF EACH YEAR.

Taxa	P	1988	1989	1990	1991	1992	1993	1994
		N = 5	16	45	40	10	20	40
All species	<0.01	61.2 $\pm$ 39.3	124.2 $\pm$ 184.3	66.4 $\pm$ 99.2	40.4 $\pm$ 40.0	38.8 $\pm$ 22.0	109.1 $\pm$ 52.2	170.8 $\pm$ 126.2
Littoral guild	<0.01	25.0 $\pm$ 27.0	30.0 $\pm$ 28.9	28.7 $\pm$ 40.0	25.9 $\pm$ 25.8	18.5 $\pm$ 15.5	65.7 $\pm$ 40.5	73.8 $\pm$ 61.6
Bowfin	<0.01	3.2 $\pm$ 3.6	0.7 $\pm$ 1.0	0.7 $\pm$ 1.0	0.8 $\pm$ 1.4	0.2 $\pm$ 0.4	2.1 $\pm$ 2.7	2.5 $\pm$ 3.0
Golden shiner	<0.01	7.2 $\pm$ 15.5	3.7 $\pm$ 7.4	9.2 $\pm$ 20.5	3.2 $\pm$ 7.0	1.8 $\pm$ 4.1	9.4 $\pm$ 15.1	24.6 $\pm$ 40.7
Coastal shiner	0.07	0.6 $\pm$ 1.3	0.4 $\pm$ 1.3	2.3 $\pm$ 7.3	0.7 $\pm$ 2.4	0.8 $\pm$ 1.3	2.6 $\pm$ 6.0	4.2 $\pm$ 11.9
Lake chubsucker	<0.01	0.2 $\pm$ 0.4	0.2 $\pm$ 0.5	3.3 $\pm$ 17.0	2.0 $\pm$ 4.5	1.0 $\pm$ 1.6	2.2 $\pm$ 4.0	3.1 $\pm$ 4.9
Chain pickerel	0.25	0.4 $\pm$ 0.9	0.8 $\pm$ 1.2	1.0 $\pm$ 1.5	0.8 $\pm$ 1.8	0.1 $\pm$ 0.3	0.9 $\pm$ 1.5	1.0 $\pm$ 2.6
Bluegill	<0.01	3.2 $\pm$ 2.5	4.5 $\pm$ 5.0	2.8 $\pm$ 4.1	5.8 $\pm$ 8.4	4.1 $\pm$ 7.3	15.8 $\pm$ 17.6	9.4 $\pm$ 13.2
Redear sunfish	<0.01	3.6 $\pm$ 5.4	1.6 $\pm$ 2.3	1.4 $\pm$ 1.9	3.0 $\pm$ 5.2	1.6 $\pm$ 0.5	8.6 $\pm$ 5.1	9.5 $\pm$ 9.9
Black-spotted sunfish	0.01	0	0.3 $\pm$ 0.8	0.6 $\pm$ 1.5	0.8 $\pm$ 3.1	2.2 $\pm$ 5.0	2.0 $\pm$ 3.3	0.7 $\pm$ 1.4
Largemouth bass	<0.01	5.6 $\pm$ 3.4	2.4 $\pm$ 2.4	4.4 $\pm$ 5.3	4.3 $\pm$ 3.4	3.6 $\pm$ 3.0	15.8 $\pm$ 12.7	11.0 $\pm$ 10.0
Yellow perch	<0.01	0.6 $\pm$ 0.5	4.8 $\pm$ 17.7	0.3 $\pm$ 0.9	0.9 $\pm$ 3.0	1.5 $\pm$ 1.8	1.9 $\pm$ 2.4	4.2 $\pm$ 7.5
Vegetation Coverage, ha		4170	4385	4800	4700	1580	323	60

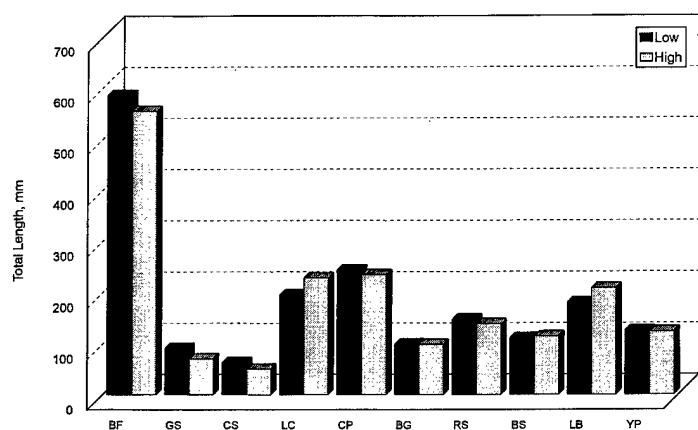


Figure 2. Mean lengths of commonly collected (>0.8% of the total catch) littoral fishes during low (1989 to 1990) and high (1993 to 1994) coverage of hydrilla in upper Lake Marion. Abbreviations for species on the x-axis are: BF = bowfin, GS = golden shiner, CS = coastal shiner, LC = lake chubsucker, CP = chain pickerel, BG = bluegill, RS = redear sunfish, BS = black-spotted sunfish, LB = largemouth bass, and YP = yellow perch.

adults to feed in open water on zooplankton (Mittelbach 1988). Although growth is a temporary response to the surrounding environment, most littoral fishes frequently collected in our study showed minimal changes in average lengths during high and low hydrilla coverage suggesting that foraging efficiency was not substantially altered. An exception was largemouth bass whose total length was statistically lower after hydrilla coverage declined, but differences between the two time periods differed by only 20 mm.

Use of grass carp to control a noxious aquatic plant in Lake Marion appeared to have minimal, if any, negative effects on the fish assemblage during the seven-year study period. However, composition of the fish assemblage will continue to respond to changes in the abundance of hydrilla depending on the level of control by grass carp. If other forms of structure remain in the upper lake, it is unlikely that a major decline of littoral fish populations will occur as found in other studies where grass carp have completely eliminated submersed aquatic plants.

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